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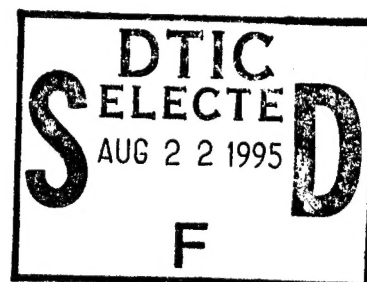
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TABLE OF CONTENTS

SF298- Report Documentation Page.....	i
Foreword.....	ii
Abstract.....	iii

Annual Technical Report - HIV Research Program

1. Preventive Vaccines.....	1
2. Behavioral Prevention.....	51
3. HIV Disease Prevention.....	95
4. Immunoregulation.....	144
5. Intervention Assessment.....	185

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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the management of the Henry M. Jackson Foundation for the Advancement of Military Medicine and are not necessarily endorsed by the U.S. Army.

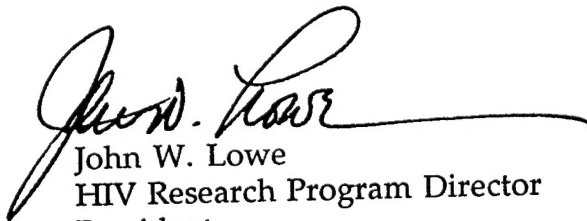
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In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.



John W. Lowe
HIV Research Program Director
President
Henry M. Jackson Foundation
for the Advancement of Military Medicine
November 30, 1994

ABSTRACT

A comprehensive HIV Research Program continued into its seventh year of scientific endeavors through a Cooperative Agreement between the United States Army Medical Research and Materiel Command and the Henry M. Jackson Foundation for the Advancement of Military Medicine. The primary mission of the program remains constant: to reduce and prevent HIV-infection among military medical beneficiaries. Five structural Program Areas produce the science to achieve this mission: Preventive Vaccines, Behavioral Prevention, HIV Disease Prevention, Immunoregulation and Intervention Assessment. Selected significant results and accomplishments during the reporting period 1 October 1993 through 30 September 1994 are listed:

1.) Preventive Vaccines:

Demonstration that HIV-1 genotypes B and E, which circulate in Thailand, are distinct neutralization serotypes and can co-infect humans.

Establishment of a partnership for efficacy testing of bivalent (genotype B and E) subunit vaccines in Thailand.

2.) Behavioral Prevention:

Utilization of a data based approach to intervention design.

Utilization of seropositive behavioral data as a key part of prevention agenda.

3.) HIV Disease Prevention:

Submission of first Phase I (Safety) gene therapy protocol.

Development of numerous innovative technologies and assays for the validation of virologic and immunologic surrogate markers.

4.) Immunoregulation:

Execution of multi-site vaccine therapy trials with nearly 2,500 patient years accrued.

Continuation of long-term follow-up vaccine therapy trials which have allowed the dissection of humoral and cellular epitopes in natural infection and the identification of additional recognition sites induced by immune manipulation.

5.) Intervention Assessment:

Collection of valuable natural history data which can be linked with longitudinal clinical samples.

Designation of Rockville facility as ACTG reference laboratory for quantitative PCR and WHMC facility as DAIDS reference laboratory for cell sorting and phenotypic analysis.

PREVENTIVE VACCINES

USAMRMC Program Area Coordinator:

HJF Scientific Director:

LTC John McNeil, MD, MC, US Army

Francine McCutchan, Ph.D.

Program Area Summary:

The primary goal of this program area is to develop and field test a preventive vaccine for HIV-1 that significantly averts disease manifestations and virus transmission. The five major areas of focus are: SIV Models, HIV-1 Model, Protective Immunity, Molecular Epidemiology and Field Site Development. Research within these areas are comprised of laboratory projects, animal use protocols and human use protocols which, in a collaborative fashion, work towards the common pursuit of an effective preventive vaccine.

Laboratory Research Projects/Human Use protocols

Project Number	Title (abbreviated)	Principal Investigator
SIV MODELS		
LRP 29	Live attenuated SIV Vaccines	Lewis
RVA19	Nef-deleted PBJ	Lewis
RVA21	Nef-deleted SIVmac239	Lewis
RVA17	Protective Immunity- "Quiescent" Infection	Lewis
LRP 30	Evaluation of Whole Inactivated Vaccines	Lewis
RVA14	Development of a More Virulent SIV Challenge stock from e11s	Lewis
LRP 31	Evaluation of Subunit Vaccines - SIV Model	Lewis
RVA3	SIV B-gal Fusion Peptide Vaccine in Freund's Adjuvant	Lewis
RVA13	SIV B-gal Fusion Peptide Vaccine/Repeat Trial	Lewis
RVA20	HIV-1 B-gal Fusion Peptide Vaccine in QS21	Lewis
HIV-1 MODEL		
LRP33	Development of HIV-1 Model in Pigtailed Macaques	Gartner
RVA15	Adaptation of HIV-1 to Pigtailed Macaques thru Serial Passage	Gartner
LRP34	Development and <i>In Vivo</i> Evaluation of SHIV	Gartner
LRP35	Cross-Species Adaptation	Gartner
RVA5	Development of HIV-2 Model in Macaques	Gartner

*RVA denotes supporting animal protocol

Laboratory Research Projects/Human Use protocols (continued)

Project Number	Title (abbreviated)	Principal Investigator
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PROTECTIVE IMMUNITY

RV87	Primary Immunoregulation of HIV-1	Artenstein
RV91A	Evaluation of Possible Dual HIV-1 Infection	Artenstein
RV107(P)	Mucosal Immunity to HIV-1	Artenstein

PRODUCT DEVELOPMENT

LRP37	Neutralization Serotyping of HIV-1	Mascola
LRP38	Evaluation of Seronegative Vaccinee Sera	Mascola
LRP39	HIV-1 Enhancement	Mascola
LRP 40	Vaccine Strain Selection/Evaluation	Mascola

MOLECULAR EPIDEMIOLOGY

LRP36	International Genetic Variation of HIV-1	Mascola
LRP 41	Intra and Intergenic Recombination between HIV-1 Genetic subtypes	Carr

SITE DEVELOPMENT - THAILAND

RV69A(P)	Behavioral Aspects of Seronegative Trials	Jenkins/Kittichatr
RV91	Natural History of HIV-1 Infection -Thailand	Carr/Markowitz
RV 99(P)	Phase I Trial of Biocine HIV SF2 gp120/MF59 Vaccine Seronegative Thai Volunteers	Nitayaphan/McNeil
RV 101(P)	Screening and Evaluation of Volunteers for HIV-1 Vaccine Trials	Nitayaphan/McNeil
RV 102(P)	Prevalence /Incidence of HIV-1 Among Recruits Royal Thai Military - Prachuap Khiri Khan	Torugsa/McNeil
RV103(P)	Prevalence / Incidence of HIV-1 Among Recruits in the Royal Thai Army	Achara/McNeil
RV 104 (P)	Feasibility Study for Vaccine Cohort Development: Clinic-Based Populations	Sirisopana/McNeil
RV105(P)	Feasibility Study for Vaccine Cohort Development: Factory Based Workers in Lampang	Krinchai/McNeil
RV106(P)	Feasibility Study for Vaccine Cohort Development: Community-Based Study in Phayao	Torugsa/McNeil

*(P) denotes protocol pending approval

I. OVERVIEW

The global spread of HIV-1 has been swift and relentless. Worldwide, ten million human beings are infected, and no country or region has been spared. The pandemic poses a potential threat to the health and well-being of military and civilian populations everywhere. Ten years of intensive research on HIV-1 and other primate lentiviruses has failed to yield a preventive vaccine of demonstrated efficacy. The correlates of protective immunity remain to be demonstrated. Vaccine development must therefore proceed empirically and by the application of principles gained from experience with other vaccines in use.

Laboratory projects, human use clinical research protocols, and animal use protocols are conducted in the Preventive Vaccines Program Area. In the laboratory, the SIV model is being used to investigate correlates of protective immunity and for comparative efficacy evaluation of vaccine types. The HIV-1 model in macaques is under development for direct efficacy testing of HIV-1 vaccines. Molecular epidemiology of HIV-1 is being tracked globally and evaluated intensively for future vaccine trial sites. The correlates of protective immunity are being evaluated using materials from human clinical research and from field studies of informative situations. Direct participation in product development is underway, both generically with the collection of data for candidate vaccine strain selection and specifically in support of the development of two vaccine candidates by companies. Infrastructure development, natural history studies, and cohort development projects are underway in Thailand where the first candidate vaccines will be evaluated within this program area. Initial evaluations of candidate vaccines are slated for early 1995 and will continue serially with different products and combinations.

During the period covered by this report several structural/organizational changes have been accomplished to further the program goals. Much of the work in the Program Area is now carried out collaboratively with other HIV Research Program Areas, maximizing the research and minimizing duplication of effort. Areas of intersection between this Program Area and the Immunoregulation Program Area have been identified and cross-program area teams have been formed to accomplish specific projects. Much of the development of both the concepts and the laboratory capabilities that support seropositive clinical trials of HIV-1 vaccines also applies to preventive vaccine trials. Many of the international human clinical reagents, some of the genetic data, and some reagents from animal model vaccine trials provide added depth to studies of immunoregulation of HIV-1. Similarly, the expertise of the Intervention Assessment Program Area in the development of quantitative validated assays for determination of viral load can, with collaborative arrangements in place, be applied to evaluation of animal models and to specialized problems of molecular epidemiology. Lastly, the Behavioral Prevention Program Area provides behavioral scientific consultation to this Program Area in the development and evaluation of methods for recruitment and retention of vaccine trial participants.

II. GOALS AND OBJECTIVES

The goal of the Preventive Vaccines Program Area is to develop and field test a preventive vaccine for HIV-1 that significantly averts disease manifestations and virus transmission. Historically, preventive vaccines have formed the best and often the only strategy to combat viral diseases. Given the potential needs of the U. S. Military to deploy personnel to HIV-1 endemic areas, an effective preventive vaccine, coupled with appropriate counseling and testing programs, is expected to be the most effective strategy to maintain readiness and strength. Specific objectives within this Program Area in support of the primary goal include the following:

- A. Evaluate worldwide genetic variation of HIV-1, describe regional distribution and prevalence of variants, investigate immunological correlates of variation, and select prototype strains for vaccine construct development and evaluation.
- B. Support development and evaluation of at least one prototype HIV-1 whole inactivated and/or live-attenuated virus vaccine candidate.
- C. Provide technological data gathered from laboratory, animal and human clinical studies to support selection of candidate prophylactic vaccines for clinical testing in at-risk individuals.
- D. Conduct detailed population definition and assessment of HIV-1 infections in a candidate test site and provide sufficient technological data to implement field efficacy trials of HIV-1 prophylactic vaccines.
- E. Utilize observational studies to assess beneficial/detrimental characteristics, both virologic and immunologic, that contribute to rapid vs. slow progression, acute primary HIV immunoregulation, the outcome of potential transmission events and the occurrence of highly exposed, persistently seronegative individuals.
- F. Develop relevant vaccination/challenge systems in nonhuman primates - including HIV-1, SIV and SHIV and establish quantifiable endpoints for protection.

III. TECHNICAL APPROACH

This Program Area is divided into five primary areas of focus: SIV Models, HIV-1 Model, Protective Immunity, Product Development, Molecular Epidemiology and Site Development. Each of these five major areas are comprised of a combination of laboratory projects, animal use protocols and human use protocols. The technical approach to this program area is detailed below; an overview of each area is presented and is followed by information on the projects and protocols.

A. SIV Model

Summary:

The Preventive Vaccines Program Area has focused its basic research activities on the only experimental model of AIDS to convincingly demonstrate an efficacious preventive vaccine: the live attenuated SIV vaccine. The objectives in this particular area are to confirm and extend this observation and to make use of reagents from protected animals to attempt to determine correlates of protective immunity. The researchers are well on their way to meeting the first objective but progress has been slower towards meeting the second objective.

The SIVmac239 virus was initially used to demonstrate protective immunity against homologous challenge. This virus generates a more protracted disease course *in vivo* than its uncloned parent, SIVmac251, and it appears that during the molecular cloning of SIVmac239 a less virulent virus was selected. The researchers have taken advantage of the reverse situation, virus PBJ14, in which a molecularly cloned virus is more virulent than the uncloned parental stock. It was postulated that superposition of the "attenuating" effect of a deletion in the *nef* gene upon a highly replicative, highly virulent strain might provide a superior protective effect because of increased initial replication and spread of the virus. Also, the homologous challenge would be acutely lethal, providing a quick (and quite dramatic) evaluation of protective efficacy. Initial indications are very positive that this experiment is going to be possible. Deletion of the *nef* gene did abrogate the acute lethality of PBJ14. The attenuated strain is still highly replicative, with virus isolation from blood persisting for weeks and then waning.

The SIVmac239 live attenuated vaccine experiment was also initiated this year. In addition to a confirmation of protection with this strain, long term natural history studies have been set up, including early sacrifice of several animals to better understand early events that generate protective immunity; the maintenance of immune function in vaccinated animals will also be investigated. None of this is being done by other groups working with this model. Comparative evaluations of the SIVmac239 and the SIV PBJ experiments should yield additional insights into common principles of protective immunity. The

researchers believe that these lines of investigation have significant potential to advance this field and this Program Area is well positioned to gather important data.

Development of evaluative capability in the SIV model has been slow. Lack of available reagents and, in some cases, of developed assays, has hampered progress. However, all of the assays that are not yet developed are being worked on with the exception of T-cell proliferation and CTLs.

The objective to be met by evaluation of whole inactivated SIV vaccine is comparative efficacy with live attenuated vaccines. Progress has been hindered by lack of a suitable production cell line of SIV origin. A suitable cell line is being pursued under contract to ABI but progress has been minimal. At this juncture, the researchers plan to reassess the importance of this component and either discontinue the work or devote the necessary resources to complete the work.

Subunit vaccine evaluations are currently limited to SIV and HIV peptides. The three remaining monkeys from the initial β -gal fusion peptide experiment are being held until the endpoint of terminal morbidity. The repeat trial of this vaccine with ten animals is troubled. If anti-SIV peptide Ab is not measurable after the 4th boost, the researchers will have to either challenge anyway or recycle controls to other experiments and abandon vaccinees. The apparent lack of immunogenicity of this vaccine is not understood at this point. The fusion peptides utilized were derived from a different production lot than used in the first experiment.

The study of HIV-1 fusion peptides is a safety/immunogenicity study conducted through a CRADA with UNIVAX, comparing Freund's with QS21 adjuvant. It is ongoing with satisfactory progress with the exception of one adverse reaction to Freund's which was noted in a pigtailed macaque.

The future viability of the Subunit vaccine project of this program (LRP31) is doubtful for three reasons; 1) efficacy evaluations cannot be easily done due to a delay in disease development is the maximum beneficial effect expected; 2) challenge stocks are not yet available for HIV-1 component and those used for the SIV component produce a protracted disease course; 3) subunit vaccines completely analogous to those used in human efficacy trials (produced in CHO cells) are not currently available. It is anticipated that this project will be terminated in the near future.

The following text describes the protocols and projects executed during the past year with progress; significant findings are also delineated.

SIV Model - Technical Approach:

LRP29 - "Live Attenuated Vaccines" -

RVA 19 - "SIVsm/PBj Δ nef Attenuated Virus Model Development"-

Project Description:

These studies were initiated in order to study the utility of a nef deleted SIVPBj molecular clone as an immunogen. SIVPBj had been shown previously to grow rapidly to high titer and to be lethal in pig-tailed macaques. The working hypothesis was that a nef deleted PBj isolate will still grow to high titer, but would not be lethal. This should lead to the development of high levels of humoral and cellular immunity in the infected macaques, while not leading to disease development. This project also proposed to determine the natural history of the infection and compare this to data previously generated in our laboratories. If the virus was found to be avirulent, additional studies would be performed to determine the ability of the generated immune response to protect the animals from subsequent challenge with virulent SIV of either homologous or heterologous genotype. Additional studies were also proposed to determine the effect of the nef deleted virus on the integrity of the infected macaques.

Significant Findings:

To date, virus stocks have been grown in human Peripheral Blood Lymphocytes (PBL), pigtail PBL, AA2 and CEMx174 cells. The virus did not grow well in primary cells, but did grow to reasonable titers in cell lines. Five pigtailed macaques were inoculated with an AA2 derived stock and all have been infected. Virus isolation was not difficult during the 4 weeks following inoculation, but became more difficult as time progressed. The animals developed detectable SIV specific antibodies by 2 weeks Post Inoculum (PI) and these titers continued to increase to high levels by 10 weeks PI. The animals showed no signs of acute disease and remained healthy during the period of study.

RVA21 - "SIVmac/239 Δ nef Attenuated Virus Model Development"-

Project Description:

With the recently reported success using an SIVmac239 nef deleted virus as an attenuated virus vaccine, the Program Area's laboratories initiated a project to study the potential use of attenuated viruses as vaccine sources. These studies were proposed to expand and further study the utility of the nef deleted SIV

molecular clone as an immunogen. This project also proposed to determine the natural history of the infection and to compare it to data generated in our laboratories and in collaboration with researchers at the New England Regional Primate Center using the non-attenuated 239 virus isolate. Additional studies would be performed to determine the ability of the generated immune response to protect the animals from subsequent challenge with virulent SIV of either homologous or heterologous genotype.

Significant Findings:

Currently, virus stocks have grown in human PBL, rhesus PBL, AA2 and CEMx174 cells. The virus did not grow well in primary cells, but did grow to reasonable titers in cell lines. Five Rhesus macaques were inoculated with an AA2 derived stock and all have been infected. Virus isolation was routine during the 4 weeks following inoculation. The animals developed detectable SIV specific antibodies by 2 weeks PI and titers continued to increase to moderate levels by 4 weeks PI. The animals showed no signs of acute disease and remained healthy during the period of study. Fifteen additional animals were inoculated with the stock and are currently being observed.

RVA 17 - "Dual Infection of Rhesus with SIV"

Project Description:

Superinfection by distantly related primate lentiviruses has been suggested as a possible source of recombinant viruses with a potential to exacerbate disease. Dual infections by closely related retroviruses possibly may not occur naturally due to viral interference or immune resistance. This phenomenon has been attributed to the blocking of the viral cell-surface receptor, or to immunity against superinfection. Superinfections by two distinct lentivirus types have been reported *in vitro* using HIV-1 and HIV-2, but only in cells that retained their surface CD4 after infection. Dual infection of a single cell by lentiviruses was found to generate viral pseudotypes with altered host ranges and phenotypic mixing between the two viruses. Dual lentivirus infections of HIV-1 and HIV-2 have been reported in humans. Dual infections with SIV isolates have also been attempted, but only for the purpose of vaccination with the first infection being of an attenuated strain. The purpose is to determine if dual infection by two virulent and pathogenic SIV isolates could occur in rhesus macaques, and if so to observe for possible alterations in disease course.

The monkeys used in this protocol were from a previous protocol (RVA 8 - Protective Effect of Neutralizing Antibody). These animals received challenge 10ID50 of SIVmne/e11s. Eight seropositive (and two seronegative) animals were utilized for this study. The seropositive animals were divided into two groups of 4 animals each, and received either a high dose (1000 MID) or a low dose (10

MID) of the SIV molecular clone 239. Two additional seronegative animals served as controls.

Significant Findings:

The monkeys were challenged with SIV239 in February 1993 and are currently being studied for disease outcome. The isolates used in this study were a molecular clone, SIVmac239 and a biologic clone, SIVmne/E11s. These isolates differed genetically by 6% in envelope and by 3% in the gag regions. As mentioned above, eight rhesus macaques were used that had been previously infected (10-12 months) with SIVmne/E11s. The monkeys had circulating SIV antibody, had been virus isolation positive, but at the time of the second challenge were isolation negative. The monkeys were subsequently challenged with either 10 ID50 or 1000 ID50 of SIVmac239. Seven of the eight animals became isolation positive within 14 day after the 239 challenge. All 7 had anamnestic responses to SIV by 7 to 21 days PI. The remaining macaque, which had the highest level of SIV-antibody prior to the second challenge, became isolation positive on week 6 PI. Four of the eight animals became difficult to coculture by 10 weeks, but only one remained isolation negative. The remaining 4 were routinely positive. No acute syndrome was detected in any of the animals with pre-existing immunity, whereas control animals had detectable antigenemia and lymphadenopathy within two weeks PI. Analysis by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) of virus loads in the peripheral blood indicated that antibody levels prior to the second challenge correlated inversely with detectable virus. The PCR and restriction analysis indicated that the virus isolated 3 weeks post infection was SIVmac239. These findings showed that rhesus macaques with varying levels of pre-existing SIV immunity to SIVmne/E11s can be dually infected with SIVmac239. The pre-existing immunity was not sufficient to block infection by the second isolate, but the level of dual infection and pattern of virus isolation appeared to be a function of the level of this immunity.

LRP30 - "Whole Inactivated Vaccines" -

RVA14 - "Titration of a *In Vivo* Passaged E11s Virus Stock (69R)" -

Project Description:

This project was conducted to test and study a rhesus passaged SIVmne/E11s virus and virus infected cells which were proposed for use in future MMCARR vaccine and pathogenesis studies. Stocks of cell associated and cell free virus were generated using cells isolated from an SIVmne/E11s chronically infected macaque. Stocks of cell free virus were produced in normal pigtailed macaque PBL. Studies with SIVe11s indicated that the disease course is more predictable, but prolonged with detectable pathogenesis not observed until

late in disease. Passage of the E11s virus has caused a more rapid disease progression (1.5 years versus >3 years) with increased pathogenesis, while maintaining its less severe acute disease. The use of this virus isolate for future vaccine studies should allow for a more rapid end point while not overwhelming the immune response generated following immunization.

Significant Findings:

Three monkeys were challenged (3-11-94) with the 69R virus grown in pigtail PBL. The free virus stock had been titered at 10^3 Tissue Culture Infective Dose 50 (TCID₅₀ per milliliter) and each monkey received a dilution of the virus stock of 10^{-3} , 10^{-4} or 10^{-5} dilution. All three animals became infected by all criteria. One animal died 5 month post challenge, and a second animal is currently showing signs of AIDS. A second group of animals were challenged (6-16-94) with dilutions of 10^{-5} , 10^{-6} , 10^{-7} of the stock. All of these animals remained virus isolation negative and antibody negative. A third group of animals was challenged (9-3-94) with dilutions 10^{-4} , 10^{-5} and 10^{-6} . The animal receiving the 10^{-4} dilution is currently virus isolation positive, while the remaining two animals are virus negative. Using the current data obtained from 9 animals, the Infective Dose 50 (ID₅₀) for the rhesus macaque of this stock of free virus is a dilution of 1×10^5 .

LRP31 - "Subunit Vaccine"

RVA 3 - "β-gal Fusion Peptide Vaccine" -

Project Description:

This study was a continuation of the development of a peptide vaccine by the HIV Research Program. The peptides were chosen for their conserved amino acid sequences and their immunoreactivity in HIV and SIV seropositive people or macaques respectively. Synthetic duplex DNA coding for these sequences from HIV-1, HIV-2 and different SIVs' were cloned upstream from coding sequences for the immunocarrier, β-galactosidase (β-gal). These vectors were then inserted into E. coli bacteria for the production of useable amounts of the β-gal peptide fusion proteins.

The initial protocol used six rhesus macaques, three received a cocktail of four peptides (SIV-88, SIV-500, SIV-584, and SIV-647) linked to β-galactosidase and three controls received β-gal alone. The monkeys received four immunizations over a one year period. The first immunization used Freund's complete adjuvant, followed two weeks later with Freund's incomplete adjuvant. The last two immunizations consisted of the peptide-β-gal cocktail alone.

Significant Findings:

The first stage of the project showed that this vaccine strategy induced high antibody titers to the four β -gal linked amino terminus peptides chosen (SIV-88, SIV-500, SIV-584, and SIV-647) as tested by ELISA and dot blot assays. In addition, two of the three monkeys receiving peptides developed detectable responses to gp120 on an immunoblot using SIVmne as the antigen. Neutralizing antibody was also detectable at a low level in all of the vaccinated monkeys. Following challenge with SIVmne/E11s all of the control monkeys (immunized with the β -gal carrier protein) became virus positive by week 4 and remained persistently positive in two cases. All of the vaccinees showed signs of limited virus replication, but for two years the virus has not been isolated from the peripheral blood of these animals. During this time two controls died of AIDS and the remaining control is showing signs of CD4+ cell loss, is routinely virus positive and has signs of immune depression, while two vaccinees have remained healthy, virus isolation negative, with no CD4+ cell loss. One vaccinee has died of SIV induced AIDS. This peptide immunized monkey, 4GI, had the lowest antiviral antibody response and had the highest viral load following challenge. Lymph nodes isolated from the vaccinated macaques were found to contain SIV DNA and one had isolatable virus. Transfer of viable lymph node cells and whole blood from these vaccinees to SIV naive macaques did not transfer detectable SIV infection. This indicates that the vaccinees, although SIV infected, have been able to inhibit virus replication and potential transfer to naive hosts. Recently the researchers have shown the plasma collected from peptide immunized macaques, when used to pretreat monkeys, protected 50% of the naive monkeys from SIV infection.

Three monkeys are being held for final outcome of disease. The three monkeys (3X7, 4GP and 4GC) are currently over four years into the study. These animals are being held for determination of immune responses following SIVmne/E11s infection. Two were vaccinated with SIV fusion peptides and are currently healthy, routinely virus negative and with circulating CD4 cell numbers stable. One animal has low levels of envelope antibodies while the other has medium to high levels of antibody to all the major viral proteins. The remaining control animal is routinely virus isolation positive, has low circulating CD4 cell numbers and high levels of anti-SIV antibodies. Two of the three controls and one of the vaccinees in this study died. The remaining monkeys are currently being used as positive controls for a number of assays being developed to study macaque humoral and cellular immunity developed following infection.

RVA13 - "β-gal Fusion Peptide Repeat Trial"-

Project Description:

This study is a repeat study of RVA-3 (Vaccines for SIV) - described above - using the same fusion peptides generated in E-coli bacteria. The peptides were chosen due to their conserved amino acid sequences and due to their immunogenicity in HIV and SIV seropositive people or macaques.

This study currently consists of 10 rhesus macaques (5 vaccinates and 5 controls). They have received 3 doses of vaccines and are awaiting their final boost and challenge. They do not have western blot reactivity or neutralizing antibody. Further analysis has been performed using a new ELISA system developed by Dr. Loomis - Immunoregulation Program Area. Alternate neutralization assays are also currently being performed. A standard ELISA to detect β-galactosidase antibodies was used.

Significant Findings:

It was determined that all of the animals developed β-galactosidase specific antibodies. The titers ranged between 10^3 to 10^5 . All of the immunized monkeys developed high titers to the four peptides. In addition, two of the three monkeys receiving peptides developed detectable responses to gp120 on an immunoblot using SIVmne as the antigen. Neutralizing antibody was also detectable at a low level in all of the vaccinated monkeys. One week following the last immunization, the animals were challenged with a dose of 100 TCID of SIVmneE11S.

RVA20 - "Immunization of Pigtailed Macaques with HIV-1 fusion Peptides"-

Project Description:

This study tested the character of the immunity generated following vaccination with conserved regions of the human lentivirus envelope. To test this pigtailed macaques were immunized with a mixture of 88, 500, 582 and 647 peptides from HIV-1. Two adjuvants were used for this study, QS-21 and incomplete Freund's adjuvant. Control monkeys were immunized with β-gal in adjuvant. The monkeys were followed for the immune response generated following immunization. The monkeys were then challenged with a macaque titrated stock of SHIV or HIV-1. The β-galactosidase alone groups were used as challenge controls.

Significant Findings:

15 pigtailed macaques received two doses of the proposed immunizations. All of the animals generated anti- β -galactosidase immunity. One adverse reaction to the immunogens or adjuvants was noted.

B. HIV-1 Model

Summary:

Serial passage of a chimp-adapted HIV-1 IIIb strain in pig-tailed macaques is apparently yielding a more highly replicative isolate and one which fairly consistently infects pigtailed macaques from cell-free viral stocks. Seroconversion of infected animals to multiple HIV-1 proteins is increasingly rapid. However, virus isolation is still sporadic. Much work remains to be done to generate a well-characterized, titered HIV-1 challenge stock. First, more consistent virus isolation and convincing persistence of virus over at least 6 months is desirable. Otherwise, time may serve as a "vaccine" for infected animals. The second problem is that it is unclear whether pigtailed macaque cells are preferred to human cells *in vitro* by the current stocks. It may be difficult to decide how to titrate the eventual challenge stock if this preference is not clarified. Another difficulty is the limited number of animals available. The researchers may need to use all of the animals that they were able to obtain for model development, leaving the actual use of the challenge stock for collaborations with other groups with more access to animals. Nonetheless, this project is moving reasonably well and will probably yield a very valuable reagent for vaccine development if carried to completion.

The objective of the SHIV project (LRP 34) is to provide for heterologous challenge among HIV-1 clades. This is a collaborative project between this Program Area and Chiron-Biocene with both laboratories working to construct Clade E SHIVs. This Program Area probably has the world's best availability of reagents for this project. The HIV-2 experiments (LRP35) have been discontinued.

HIV-1 Model : Technical Approach:

LRP33 - "HIV-1 Adaptation to Pig-tailed Macaques"-

RVA15 - "HIV-1 Models in Primates"-

Project Description:

A total of 12 monkeys are currently on study, 10 pig-tailed macaques, 1 cynomolgus macaque and 1 cynomolgus/pig-tail hybrid; animals are housed at Southern Research Institution in Frederick, MD.

The desirability of an HIV-1 model of infection in monkeys is obvious. The necessary first objective of this project was to identify and characterize an HIV-1 isolate able to establish persistent infection *in vivo* for at least six months as demonstrable by frequent isolation of infectious virus from peripheral blood cells and maintenance of a well-developed anti-viral antibody response to multiple viral proteins. Following identification of a candidate isolate via *in vitro* studies, four pig-tailed macaques were inoculated using autologous cells exposed to the virus *in vitro*. Although this initial *in vivo* experiment was severely compromised because two of the four animals had to be sacrificed prematurely, being infected with Simian Retrovirus type D (SRV), the researchers were able to demonstrate long-term persistent infection in one animal as evidenced by the continued presence of viral genomes in peripheral blood, maintenance of an anti-HIV antibody response and occasional virus isolation. An isolate recovered from this animal at week 61 manifested several biological differences important to the successful development of this model. It replicated to significantly higher titer than the parental virus and was more syncytia-inducing. Most importantly, it infected pig-tailed macaque T lymphocytes *in vitro* considerably better than the parental virus, suggesting that some degree of adaptation has taken place. It was reasoned that multiple *in vivo* passage might provide further enhanced adaptation, a hypothesis currently being tested. The week 61 isolate was passaged cell-free into naive pig-tailed macaques. Initially, three animals received a human cell-propagated stock and three received a pig-tailed macaque cell-propagated stock. The titer of the human cell stock was approximately 18 times higher than the macaque-derived one, but researchers considered it possible that macaque cell-propagated virus might be more able to establish a good infection in the animal, and hence, required experimental determination. Additionally, a previously-exposed cynomolgus monkey and a naive cynomolgus/pig-tailed hybrid macaque were also inoculated with the human cell stock.

Significant Findings:

A different pattern of infection/immune response was observed among each of the three human cell stock recipients. One animal seroconverted by week 8 with demonstrable antibody to HIV-1 p17, p24, gp120 and gp160 as determined by western blot; antibody to gp41 was first detectable at week 13. These antibody responses have continued at maintenance or increased levels up to the present (week 38). HIV-1 was isolated from a biopsied lymph node of this animal at week 2 and from the peripheral blood mononuclear cells at weeks 3, 6 and 14.

A second animal was virus isolation positive from lymph node at week 2 only and only developed an antibody response to gp160, being first detectable at week 19. Following recent reinoculation with an isolate obtained from one of the other second passage recipients, a humoral response developed very rapidly, obviously suggestive of prior exposure. By week 3 the animal had antibody to p17, p24, gp41, 120 and 160. (Virus isolation studies are still ongoing.)

The third human cell stock recipient developed antibody to gp41 and 160 at week 8 and antibody to p17 at week 23, a pattern indicative of a response associated with virus replication as opposed to virus-as-antigen. However, this animal has not been virus isolation positive thus far.

In addition, the cynomolgus monkey recipient developed strong antibody responses to p17, p24 and gp160, but no infectious virus has been recovered from peripheral blood cells. The cyno/pig-tail hybrid developed some antibody to p24, but has continued as virus isolation negative.

While at least some of the animals that received the human cell stock clearly became infected, none of the pig-tail cell stock recipients appeared to have been infected as monitored during the first 12 weeks following inoculation. Therefore, these animals were rechallenged at that time with the human cell stock. Two of the three definitely became infected as evidenced by the recovery of infectious virus at weeks 5 and 9 (both animals) and week 13 (one animal). Both developed good humoral responses to gag and env proteins with appropriate time kinetics. The third animal did not respond, presumably since much of the viral inoculum was accidentally introduced subcutaneously, rather than intravenously.

Unfortunately, there was obviously considerable variation among animals with respect to susceptibility. However, the human cell stock was clearly able to infect several animals, in contrast to the macaque cell stock, most likely because the titer of the macaque cell stock was too low. The recovery of infectious virus, coupled with the magnitude and breadth of the antibody responses observed, indicated that the week 61 isolate manifested, *in vivo*, some degree of macaque adaptation. Very recently, a third passage experiment was initiated, utilizing an highly replicative isolate recovered from one of the second passage animals. The

first recipient of this virus, a juvenile animal, was isolation positive at day 5 and week 6 and had detectable antibody to p24, gp41, 120 and 160 by week 6.

Two other objectives important for optimal development of this model are currently being pursued. One involves the identification of molecular determinants associated with adaptation, and the other involves the demonstration of HIV-1 neutralizing antibodies in sera taken from the infected animals. The first of these objectives will hopefully accelerate the development of the model and extend its applicability to virus strains relevant to our proposed clinical trials. The second objective is obviously important both for the demonstration that the antibodies resulting from infection of these animals have measurable biological function, as well as for the provision of a starting ground to begin the development of virus neutralization assay systems that can be used in parallel, and perhaps interchangeably, for both animal studies and human clinical trials involving evaluation of the same vaccine(s).

Towards the identification of genetic changes present in the week 61 isolate, the researchers have succeeded in developing several molecular clones containing full-length gag, nef and gp120 genes. These are currently being sequenced. Also, in recent neutralization experiments using three different target cells—normal human T cells, neoplastic human T cells (H9) and normal pig-tailed macaque T cells—virus neutralization of HIV-1 IIIB in H9 cells was demonstrated with both of the HIV-1-infected macaque sera assayed. Somewhat surprising, however, these same sera, again using H9 cells, could not neutralize either the week 61 isolate used to infect these particular animals, or the isolate used in the first passage experiment. (The first passage virus was an isolate recovered from a chimpanzee persistently infected with the IIIB isolate.) No neutralization with the animal sera was observed using normal human or macaque T cells. Such a phenomenon is reminiscent of those observed with patient sera using different target cells and virus isolates. It appears, then, that our macaque model might provide for the elucidation of the mechanisms involved in this phenomenon, particularly as they relate to virus entry into cells. Given that the HIV research field at large currently has no real understanding as to why one cell system yields a virus neutralization titer of 1:10,000, while the same serum titers at 1:20 in a different cell system, our work with this macaque model might prove to be tremendously important to our ability to identify correlates of protective immunity as well as develop standardizable assay systems to detect these correlates.

LRP 34 - "HIV-1 Models - SHIV for Cross Challenge" -

Project Description:

To aid our identification of putative protective vaccines suitable for clinical evaluation in Thailand, additional test systems are required. In particular, our ability to evaluate HIV-1 gp120 subunit vaccines in animals is currently limited to the chimpanzee model, the analog SIV model and possibly the HIV-1/pig-tailed macaque model. At this time, both the chimpanzee and pig-tailed macaque models are limited to only certain virus strains and are also frequently suboptimal with respect to readily detectable viral persistence. The SIV model, while an excellent analog system, does not lend itself to the direct evaluation of HIV-1 products. As one alternative, the researchers plan to construct infectious SIV/HIV chimeric viruses bearing gp120 genes molecularly cloned from Clade E isolates recovered from individuals in Northern Thailand.

Significant Findings:

Candidate cloned envelope genes were selected, including one corresponding to that already chosen for development and production by Biocine as a recombinant gp120 vaccine. Also, Dr. Paul Luciw's SHIV (HIV-1SF-2 gp120 on an SIVmac 239 backbone) was procured for this work. (Unlike some of the other SIV/HIV chimeras currently available, this construct has shown to establish good persistent infection in rhesus macaques *in vivo*.) Shortly, molecular construction and genetic verification of the hybrid viruses will be initiated, followed by *in vitro* evaluation of their biological activity. *In vivo* studies will then proceed. Additionally, other Clade E isolates (obtained from investigators at Johns Hopkins) are being examined with respect to biological phenotype; the ultimate goal being identification of additional isolates appropriate for development as prototype chimeras and/or macaque-adapted strains.

LRP 35- "HIV-2 Model/Cross Species Adaptation"-

RVA5 -" HIV-2 of Rhesus Monkeys "-

Project Description:

A total of 8 rhesus macaques were utilized for this project; the animals were housed at SRI/FRC.

While it appears that HIV-2 is less pathogenic in the human host than HIV-1, some individuals infected with HIV-2 do develop immunodeficiency disease and die. Previously, the Principal Investigator recovered an HIV-2 isolate from a Portuguese seaman who succumbed to immunodeficiency disease. (His

African wife, who was infected with both HIV-2 and HTLV-I, was clinically healthy at the time.) This isolate behaves very aggressively *in vitro* and infects neoplastic cells as well as normal T lymphocytes and monocyte/macrophages. Almost five years ago, this isolate, as well as another (the Swedish 6669-SBL), was used to infect juvenile rhesus macaques. All animals initially became virus infected. However, the infection ultimately waned in some as evidenced by the inability to recover infectious virus from peripheral blood and the loss of a humoral immune response. Two of three animals inoculated with the Portuguese virus, and one of four inoculated with the Swedish virus, remained persistently isolation and antibody positive. Because this project, as originally conceived, was determined to be tangential relative to the critical path of vaccine development, a decision was made to discontinue it. So as to make optimal use of the animals and perhaps gain insight into issues of re-exposure and protective immunity, the researchers chose to explore a previously observed rechallenge phenomenon using the "negative" animals. These animals had all been virus isolation and antibody negative for at least 18 months prior to reinoculation. All members of this group were rechallenged with the Portuguese virus. Each animal underwent lymph node biopsy at the time of reinoculation to determine virologic status beyond the blood compartment. Included was a macaque from another protocol previously inoculated first with the Swedish, then the Portuguese virus. This macaque also had been virus isolation and antibody negative for some time. The animals were sacrificed at days 8, 13 or 14 post reinoculation and lymphoid tissues were removed for evaluation. Virus isolation studies have been completed; histologic evaluations for viral mRNA and protein expression, as well as serologic evaluations, are in the preliminary stages of analysis.

Significant Findings:

(1) As evidenced by virus isolation and the detection of viral mRNA in the Time0 lymph node specimen, there was occult infection, as well as ongoing virus replication, in the one seemingly negative animal originally inoculated with the Portuguese isolate.

(2) In this animal, but not the others, an "amnestic virologic response" was observed. This was indicated by the rapidity with which virus could be detected by culture and the magnitude of the expression. These parameters suggest a significantly higher viral load in this animal at the time of sacrifice than expected. Moreover, the extent of anti-viral immunostaining observed in lymph node tissues taken at sacrifice is compatible with the idea of virus-reactivation following re-exposure.

(3) All other animals became infected upon rechallenge except the one previously inoculated with both viruses.

(4) In all but one isolation-positive animal, virus was recovered from peripheral blood and lymph nodes, but not thymus or bone marrow. In the exception, virus was recovered from lymph nodes from all three sampled sites, but not from blood. Isolation from spleen was variable. A developed picture of the meaning and explanation for the findings awaits the additional information collection. It is clear, however, that HIV-2 infection, at least in macaques, can be present as a long-standing (several years) occult infection in which no virus-infected cells circulate and no anti-viral antibody is made.

C. Protective Immunity

Summary:

The breadth of protective immunity afforded by vaccines based on a single strain of HIV-1 is unknown and difficult to approach without a fully developed HIV-1 animal model. The following series of goals were set to develop knowledge in this area:

1. Establish that dual infection with HIV-1 of different clades is possible *in vivo*, using ideal epidemiological settings;
2. Develop tools to screen for dually infected individuals in populations;
3. Establish that serial infection with different clades can occur; and
4. Determine the immunologic, clinical, and epidemiological aspects of dual infection.

Goal (1) has been attained through RV91 A and goal (2) is under development in this Program Area and the Intervention Assessment Program Area; the immunologic assessment of dually infected individuals is also underway collaborative with the Immunoregulation Program Area. Work continues with proposed protocols to fulfill goals (3) and (4):

RV87 - "Immunoregulation and Pathogenesis of Symptomatic, Primary HIV-1 Infection" has been approved and patients are being enrolled. This study's objective is to determine which aspects of anti viral immunity come into play in the early clearance of HIV-1 after seroconversion. These may provide early evaluative criteria for future vaccine trials.

RV91A - "Dual HIV-1 infection" has been established with this addendum to the Thai Natural History Study. Nonrandom analyses of a group of these dual reactors (using "monoreactors" as controls) were performed

using a variety of molecular techniques and serologic assays to validate the concept of dual HIV-1 infection with distinct genetic subtypes.

RV107 - "A Measurement of HIV-1 Specific Immunological Responses in the Female Genital Tract" is pioneering in its approach and is moving rapidly through the approval process.

Technical Approach:

RV91A - "Dual HIV-1 Infection "-

Project Description:

Laboratory studies have been derived from observations in the RV91 study "The Natural History of HIV-1 Infection and Disease in Thailand". Some specimens were noted to show dual reactivity in subtype-specific PCR typing procedures. Nonrandom analyses of a group of these dual reactors (using "monoreactors" as controls) were performed using a variety of molecular techniques and serologic assays to validate the concept of dual HIV-1 infection with distinct genetic subtypes.

There are two prevalent subtypes of HIV-1 responsible for the epidemic in Thailand: B and E. These are readily distinguished by analysis of env gene sequences, as they diverge by 25-30% in this region. The researchers therefore made use of subtype-specific sequences in an overlapping region of gp120 to design B and E specific probes to screen molecular clones from individuals that were dually reactive on either PCR typing or serologic typing screens. Clones were subsequently sequenced to provide definitive proof of dual infection.

Significant Findings:

Two, epidemiologically unrelated individuals were identified who each harbor HIV-1 of distinct genetic subtypes, i.e. dual infection. HLA typing of the DNA was performed to exclude patient "mixing" as an explanation for our results. One of the patients showed serologic responses to antigens from both subtypes, and both subtypes were identified in DNA derived from co-cultured material in the other patient. Taken together, these results suggest that both viral subtypes can be replication competent and immunogenic in dual infection.

These data are the first to show dual infection with HIV-1 in infected individuals. These findings are potentially important with regard to vaccine strategies, viral evolution and viral pathogenesis. A manuscript on these findings has been submitted to Journal of Infectious Diseases and is currently in review there. The findings will be presented at the 34th annual Interscience Conference on Antimicrobial Agents and Chemotherapy/Infectious Diseases

Society of America meetings in Florida in October 1994 and the 7th annual NCVDPG meeting in Virginia in November 1994.

A panel of specimens from serologically-identified dual reactors are currently being evaluated to confirm the presence of both subtypes. Future studies are in progress to assess the following issues: 1) the performance characteristics of V3 serotyping as a screen for dual infection; 2) the prevalence of dual infection in Thailand; 3) the clinical implications of dual infection; and 4) the presence of recombinant virus as a result of dual infection.

RV87 - "Immunoregulation and Pathogenesis of Symptomatic, Primary HIV-1 Infection"

Protocol Description:

The primary protocol objectives are 1) to characterize and correlate immunologic and virologic "events" in the early period after infection with HIV-1, and to follow these prospectively; and 2) to establish a "bank" of properly stored reagents for potential use in future studies. This protocol focuses on the first few months of infection, during which time high levels of viremia and replication are controlled, to some extent, by the host. Understanding the host and viral responses during this period may be crucial towards defining beneficial immune responses. For this reason the protocol involves twice weekly blood draws over the first month, and then gradually tapers off over the ensuing three months.

Technical Approach:

Aliquots of PBMC, serum and plasma, as well as tissue specimens (where indicated for medical purposes) are stored on each patient. To date, there have been over 40 patient visits related to this protocol. An investigator meeting is planned for the late Fall.

Significant Findings:

This protocol began enrolling patients in February 1994; the original expectation was for 3 to 5 patients per year; currently 4 individuals are enrolled. Laboratory work to date: CD4+ T cell lines have been set up on three of the four patients, and epitope mapping of T cell responses is planned. Additionally, genetic analysis of serial specimens from each patient, viral burden, and humoral epitope mapping will be performed on each patient. This work will be done in collaboration with the Immunoregulation Program Area.

Number of Patients Enrolled: 4

Sites: Walter Reed Army Medical Center (WRAMC), Washington D.C.
William Beaumont Army Medical Center, El Paso, TX
National Naval Medical Center (NNMC), Bethesda, MD
Fitzsimons Army Medical Center, Aurora, CO

RV107-"A Measurement of HIV-1 Specific Immunological Responses in the Female Genital Tract" -

Project Description:

The study is a collaborative effort between this Program Area and the Immunoregulation Program Area and will enroll patients at WRAMC and NNMC. The objectives of the study are 1) to determine the feasibility of measuring HIV specific humoral responses at mucosal sites; and 2) to compare these measurements across multiple mucosal compartments within infected women. As mucosal sites are important in the transmission and putatively in the pathogenesis of HIV infection, it is likely that the induction of local immune responses will be required of any prophylactic vaccine, whether given via mucosal routes or systemically. This raises the more fundamental concept of the common mucosal immune system, i.e. do immune responses measured at "remote" sites reflect those at sites of viral transmission? The study is designed to address these issues.

Technical Approach:

Women who meet the criteria for enrollment and provide written consent will undergo specimen collection from five sites: endocervical, vaginal, nasopharyngeal, parotid saliva and peripheral blood. Total IgG, IgA, and secretory IgA (S-IgA) will be measured on each secretion from each subject using capture ELISA methods. Subsequently, HIV-1 specific Ig's will be measured using a direct ELISA with oligomeric gp160 as the antigen. Additionally, viral culture and neutralization studies will be performed on selected samples (i.e. those with the highest antibody activity).

Analyses will be performed to determine whether the measured responses for each Ig type correlate between the various mucosal compartments from each subject and within each subject. Additional analyses will be performed using collected epidemiologic and clinical data to assess the relative effects of these parameters on the humoral responses at each compartment. This clinical research study will be funded through a grant from the Defense Women's Health Research Program and is expected to be fully approved by 15 November 1994. It is anticipated that the study will be completed within one year of approval. It is also anticipated that the information and samples obtained from this study will

be used in the design of future studies to expand and broaden the findings and to address further issues of importance with regard to vaccine design.

Significant Findings:

This clinical research protocol was approved by the Retrovirus Clinical Review Committee (RCRC) and is currently before Tri-Service Human Use Committee.

Sites: Walter Reed Army Medical Center (WRAMC), Washington D.C.
National Naval Medical Center (NNMC), Bethesda, MD

D. Product Development

Summary:

Neutralization Serotyping of HIV-1 has produced significant new data in the period covered by this report. Use of an infectivity reduction format has simplified data collection and provided the assay with more range. Neutralization serotype E now includes a wide range of genotypically diverse isolates within clade E from Thailand, Indonesia, Central African Republic. Isolates from AIDS patients in Thailand, including those with atypical V3 loop "crowns", maintain neutralization serotype E. Neutralization serotype B is also maintaining the congruence of genotype and immunotype, since diverse isolates from Brazil, Indonesia, and Thailand of genotype B maintain their B serotype. Surprisingly, isolates of genotype F from Brazil belong to neutralization serotype B. This project is slowed down only by lack of reagents, particularly sera from clades A, C, D, F, G, H.

The evaluation of seronegative vaccinee sera is proceeding collaboratively with the Immunoregulation Program Area. Availability of reagents regulates progress.

HIV-1 enhancement has been difficult to demonstrate *in vitro*. The researchers are exploring the ELAV model, where it has been demonstrated *in vivo*. Reagents from dually infected individuals (HIV-1 of different clades) may be evaluated under this project. Vaccine Strain Selection/Evaluation project has been initiated to support development of whole inactivated vaccine with ImmunoAg. The goal is to provide ImmunoAg a Thai E viral strain that:

- a. is representative of the genotype,
- b. retains envelope,
- c. meets production requirements, and
- d. can be adjuvanted with retention of functional immunogenicity

Adaptation of Thai E strains to continuous T-cell lines is being explored. Envelope retention and neutralization serotype will be investigated and followed by sequencing. Progress is more rapid than initially expected for the adaptation step.

Product Development -Technical Approach:

LRP37 - "Neutralization Serotyping of HIV-1"-

Project Description:

Serotyping of international isolates has been extended from B and E viruses to other genotypes and has been verified in several PBMC assay formats. Type C viruses from Africa appear to be a distinct serotype. In contrast Brazil F viruses are serologically indistinguishable from type B viruses. The limits of genetic diversity that define neutralization serotypes is also being evaluated. Genotype E viruses from Indonesia and Africa appear to be serologically similar to Thai E. Likewise, U.S., Thai, Brazil, and Indonesian genotype B viruses are all serologically similar. Identification of epitopes which define neutralization serotypes is planned in collaboration with the Immunoregulation Program Area. Studies include serum depletion experiments and testing of immunogenicity of oligomeric (vs monomeric) gp160.

Significant Findings:

Multiple neutralization serotypes of HIV-1 exist. Experiments which would identify the epitopes which define serotypes are in progress.

LRP38 - "Evaluation of Seronegative Vaccinee Sera"-

Project Description:

Studies of sera from humans immunized with recombinantly expressed gp120 or gp160, revealed that the serum contained high titers of neutralizing Ab against the homologous lab strain, but lacked neutralizing antibody activity against several field isolates from the U.S. via the NIH AIDS Vaccine Clinical Trials Network.

Significant Findings:

A total of 64 sera representing 6 different env subunit products were evaluated. Human vaccinee sera from octomeric peptide products and from immunization with virus like particles were tested and found to give a similar result. Sera from multiple vaccine products evaluated to date demonstrate strong levels of functional antibody but do not neutralize primary HIV-1 isolates.

LRP39 - "Protective Immunity - HIV-1 Enhancement"-

Project Description:

A two year \$400,000 contract was awarded by the USAMRMC to Dr. Ron Montelaro to evaluate vaccine related protection and enhancement against a equine lentivirus (ELAV). This data may have important ramifications for HIV vaccine design. *In vitro*, cross-clade neutralization studies using sera from HIV-infected persons has not shown significant enhancement of viral infectivity of PBMCs. Limited studies of vaccinee sera has revealed no evidence of enhancement of viral infectivity against homotypic or heterotypic viruses.

Significant Findings:

In vitro studies for HIV enhancement have been mixed. In our laboratories, the effect was not seen using an increase in viral TCID₅₀ in PBMCs as a measure of enhancement. *In vivo* modeling in ELAV may provide insight into the type of vaccine products that protect or enhance in lentiviral diseases and may provide insights into the correlates of vaccine protection and enhancement.

LRP40 - "Vaccine Strain Selection/Evaluation"-

Project Description:

- a. Chiron gp120 studies - In order to facilitate pre-clinical and Phase I vaccine studies laboratory adaptation of Thai E viruses is required. Collection and screening of multiple Thai viruses is underway to find isolates suitable for adaptation. This work will be done concomitantly at the HIV Retroviral Laboratory and Chiron.
- b. Inactivated Whole Virus Vaccine Development (IWVV) - An IWVV also requires adaptation of a Thai E virus to a cell line. As above, such experiments have just been initiated. Multiple cell lines are being evaluated for growth characteristics that would be suitable for IWVV development. In collaboration with the Immunoregulation Program Area, studies of env retention, adjuvanting and immunogenicity are underway.
- c. Development of an immunogen with ability to neutralize primary isolates - experiments are underway as described in LRP37 (Neutralization serotyping). In addition, as new products are developed and are in pre-clinical testing, collaborations with vaccine manufacturers will continue to evaluate antibody response

in vaccinees. Some new strategies of interest are vaccinia expressing env, gag, pol; VLPs including neutralizing gp41 (ELDKWA) epitope and DNA inoculation with env gene.

Significant Findings:

Studies are in progress.

E. Molecular Epidemiology

Overview:

The goals of this section are to continue global surveillance of HIV-1 genotypes, to provide field-ready tools for genotyping, and to investigate the contribution of recombination to HIV-1 evolution, adaptation, and spread.

A total of 72 new HIV-1 strains have been received for genetic analysis, and 26 have been genotyped to date. Very significantly, our data establish the wider presence of HIV-1 genotype E in Southeast Asia and document the spread of this virus to Indonesia and to Uruguay by returning U.N. peace keepers who served in Cambodia. In light of the explosive spread of genotype E in Thailand, these are disturbing findings. Also, the Navy Ports of Call study has yielded at least 1 genotype E infection in a returning sailor. These efforts need to be magnified several fold due to their importance in predicting the future course of the global pandemic. A collaborative Navy unit is a welcome asset.

An effort to field genetic typing capability for genotypes B and E has been complicated by the discovery of dual infections. The goal is to establish the limits of test sensitivity and specificity and, secondarily, to distinguish lack of specificity from dual infection for selected samples. This area, too, has required additional attention and resources. A collaborative project is ongoing with the Immunoregulation Program Area which uses V3 peptide serology and holds promise but its extendibility to other clades doubtful.

Investigation of HIV-1 genetic recombinants has an initial technical requirement: recovery of full-length (or nearly so) HIV-1 genomes. The two possible approaches to this technical requirement are being pursued co-operatively with the Immunoregulation Program Area. Two very significant advances have been:

1. Immunoregulation Program Area development of CD8-depleted PBMC cultures yielding higher viral titer and increased HIV-1 proviral DNA content. This should greatly facilitate library construction in phage lambda.

2. Extended PCR is working extremely well to generate nearly full-length HIV-1 genomes from multiple clades. Some technical difficulties remain in cloning them. Once overcome, a wealth of data will be in reach, including:
 - a) genetic organization of HIV-1 of multiple clades (unknown for clades C, E, F, G, H)
 - b) nature of apparent intra-clade recombinants (A/E, A/D to begin)
 - c) generation of infectious molecular clones from multiple clades (none available from clades A, C, E, F, G, H).

Molecular Epidemiology - Technical Approach:

LRP36 - "International Genetic Variation of HIV-1"-

Project Description:

This protocol is a collaborative effort between the Navy Research and Development Command and the HIV Research Program, is designed to collect HIV-1 strains from geographically diverse regions of the world. These strains will then be characterized genetically and antigenically in order to track the changes in the virus over time and space, information crucial to the design of an effective vaccine.

There are multiple genetic subtypes of HIV-1. If these subtypes represent antigenically distinct groups, a broadly effective vaccine may have to generate an immune response to numerous subtypes. Definition of HIV-1 genetic and immunologic variation over time and space will make an important contribution to HIV-1 vaccine development. To arrive at a more complete understanding of viral variation, a large number of complete specimens (virus with matching serum) from international locations will be analyzed. The impact of international peace-keeping forces in contributing to the genetic mixing of the strains of virus will be assessed.

The Objectives of this Project are:

- a. Establish a comprehensive collection of HIV isolates and associated serum from infected individuals from international locations. This collection is not designed to be exhaustive but will concentrate heavily on regions with new or emerging HIV epidemics, locations of peace-keeping involvement or locations with potential for vaccine cohort development.

- b. Characterize these viruses both genetically and antigenically.

Significant Findings:

To date, a total of 27 specimens have been received. The geographic distribution of the specimens are as follows:

Country	Military/Civ	Comments	#of Spec	Genotype B	/ E	Not yet determined
Uruguay	Military	Deployed to Cambodia	6		4	2
	Military	Not deployed	6	6		
Indonesia	Military	Deployed to Cambodia	7	0	7	0
	Civilian	Not deployed	2	1	1	0
Korea	Civilian	Seropositive, healthy	6			6
Dominican Republic	Civilian	Mixed	10			10
Navy Port of Call	Military	Infected Overseas	35	6	1	28
TOTAL			72	13	13	46

As part of an ongoing effort to track the HIV-1 epidemic through molecular genetic analyses, specimens have been obtained from U.N. Peacekeeper soldiers from Uruguay and Indonesia following deployment to Cambodia. The Uruguayan samples, consisting of PBMC and sera from 12 subjects (6 of whom were deployed) were obtained in collaboration with the Uruguayan military, and the studies were performed in our laboratories. The Indonesia samples, consisting of PBMC and sera from 9 subjects (7 of whom were deployed) were analyzed in the laboratories at Naval Medical Research Institute. Those who deployed appear to have been infected in Southeast Asia. Samples from Korea and the Dominican Republic have not yet been analyzed.

The methods of analysis were similar for the two sample sets:

- 1) V3 serologic subtyping was performed using subtype B and E peptides in collaboration with the Immunoregulation Program Area;
- 2) A 630 bp fragment of gp120 from each subject's PBMC (including a portion of C2 region, V3, V4, V5) was PCR amplified, molecularly cloned and sequenced. The sequences were subsequently analyzed using DNASTar software. The analysis is not yet complete.

Preliminary analysis shows the serologic typing to correlate loosely with that obtained via DNA sequencing. Four of the six Uruguayan peacekeepers infected in Southeast Asia are infected with HIV-1 subtype E, whereas all six who were infected in Uruguay have subtype B. For the Indonesian panel, all those who deployed were infected with subtype E; of the two that did not deploy, one was infected with subtype E and one with subtype B.

These data are potentially important with regard to global HIV-1 vaccine strategies. The researchers have documented the introduction of a novel genetic subtype of HIV-1 (subtype E) into a previously (and putatively) "naive" population (i.e. Uruguay). Additionally, these findings point out the potential for the geographic "mixing" of HIV-1 subtypes as a result of ongoing geopolitical global forces. This could result in a significant broadening of the epidemic in terms of genetic variation which underscores the need for a broadly effective vaccine.

This protocol was approved by the NMRDC Human Use committee in July 1993 and sample collection began September 1993.

LRP41 - "Recombination Between HIV-1 Genetic Subtypes"-

Project Description:

Genetic analysis has revealed that HIV-1 can be divided into at least 7 (A-G) subtypes or clades. In some cases the subtype classification for a given isolate differs depending on which part of the genome is analyzed. This indicates that some viruses might be recombinants between two subtypes, or that the isolate is a mixture of two clones from different isolates. The following considerations must be addressed:

- Genetic recombination between HIV subtypes may be a relatively common phenomenon.
- Rules and mechanisms for recombination events generating viable genomes are not known, but could be studied by sequence analysis of cloned full-length viruses.
- Full-length clones can be generated either by phage genomic library cloning or PCR amplification of entire genomes followed by cloning.
- For several HIV subtypes, full-length clones are not available, and the full genetic structure of the virus remains unknown. Full length cloning would provide the means to generate this information.

- Development of methods to detect recombination would benefit vaccine development studies, by giving means to screen for naturally circulating recombinant viruses.

Given these considerations, the project objectives are:

- a. Determine whether the above described viruses are recombinants, or whether they represent double infections.
- b. Develop methods for the cloning and sequencing of whole HIV-1 proviral genomes.
- c. Study the recombination events, and determine the mechanisms of production of viable recombinants.
- d. Develop methods of rapidly screening for double infections or recombinant viruses.

Significant Findings:

The study was initiated in June 1994, and is in the preliminary stage. However, all needed HIV isolates were available, and successful amplification of full genomes was achieved. Obtaining representative full length clones from different subtypes is anticipated within the end of the year 1994. The sequencing and development of recombination screening is planned to span over the years 1995 and early 1996. Completion of the project is anticipated by July 1996.

The researchers found that primers and amplification procedures from different clades were able to be successfully devised for the amplification of full-length HIV provirus. The products will be cloned in either plasmid or phage vectors, sequenced and assessed for viability by transfection assays.

In the future, PCR amplification together with sequencing and heteroduplex mapping (HMA) of whole viral genomes will be applied to selected candidate virus isolates of known differing subtype in gag and env genes, to determine whether the viruses are recombinants. Found true recombinants will be fully sequenced to determine the potential recombination sites. Sequence analysis will be used to compare sites of recombination, to try to elucidate whether patterns of preferred sites exist. Attempts will also be made to artificially generate recombinant viruses, either by cotransfection or genetic engineering, with the goal of determining which recombinants are infectious, or not defective. Heteroduplex mapping from different parts of the genome is to be developed as a rapid way of screening for recombinants. Long PCR will also be applied to obtain a type collection of representative clones from all genetic subtypes of the virus.

F. Development of Vaccine Evaluation Site: Thailand

Summary:

The objectives of this component are

- a) Develop an infrastructure to support natural history and Phase 1 evaluations of Preventive Vaccines.
- b) Develop cohorts for Phase II and III efficacy trials.

The ongoing natural history study, RV91 - "The Natural History of HIV-1 Infection and Disease in Thailand" will provide important baseline data for future vaccine trials. This study represents one of the most comprehensive and systematic evaluations of the clinical course of HIV infection with a non-B genotype virus ever conducted in a developing country. Development of laboratory assays associated with this protocol has been slow but additional resources are now being provided.

The most significant development in this area is the strong new partnership being formed with Chiron Biocine for evaluation of a genotype E gp120 vaccine in Thailand. This is virtually the only avenue to generate data pertaining to vaccine efficacy in humans in the foreseeable future, and the ability of our program to successfully complete this evaluation will directly affect the future course of HIV-1 vaccine research. The immediate goal is to move the initial genotype B protocol (RV 99 - "A Phase I trial of Biocine HIV SF2 gp120/MF59 Vaccine in Seronegative Thais") through the evaluation process, including approvals in Thailand, and then execute this Phase I trial. The second objective is to facilitate follow on with the genotype E vaccine, and then utilize a bivalent vaccine in a trial as soon as possible.

ImmunoAg, a pharmaceutical firm, has expressed interest in producing a whole inactivated genotype E vaccine. Participatory development will be needed to bring this vaccine along, and the researchers are collaborating with the Immunoregulation Program Area to tackle the many inherent problems of this approach with HIV-1. A series of meeting with ImmunoAg in the future months will be required to solidify plans.

Cohort development for Phase III trials has been launched with the development of several protocols currently in the approval process. The energy devoted to this aspect of the program should be apparent from the sheer volume of paper coming through the system.

Development of Vaccine Evaluation Site: Thailand - Technical Approach:

RV70 - "Prevalence and Incidence of HIV Infections in the Royal Thai Military "-

Protocol Description:

This protocol was a study designed to provide nationwide seroprevalence and limited seroincidence data on the epidemic among recruits serving with the Royal Thai Army in Thailand. The objectives set forth were:

- a. Assess temporal, geographic and demographic correlates of HIV-1 infection among young men entering service with the Royal Thai Army (RTA) nationwide.
- b. Directly measure the rate of incident HIV-1 infections among young Thai men serving with the RTA during their two year service obligation in the Northern and Bangkok regions.

Technical Approach:

This protocol collected demographic information and HIV test results on young men entering service with the Royal Thai Army (RTA) nationwide. HIV test results were collected biannually among young Thai men serving with the RTA during their two year service obligation in the Northern and Bangkok regions.

Significant Findings:

This study was completed during this program year. The data collected represents one of the most comprehensive evaluation of HIV-1 prevalence and incidence ever conducted in the developing world. More than 117,000 prevalent cases and more than 17,000 incident cases were evaluated from a geographically distributed sampling in Thailand.

The incidence of HIV-1 infection in some provinces in Northern Thailand during this two-year study exceeded 1%, while the prevalence on entry into service exceeded 5% in several provinces. These data underscore the urgency of vaccine development for Thailand and provide data directly applicable to the design of future vaccine trials.

Volunteers studied:

Prevalence: 117,861
Incidence: 17,606

Site: Armed Forces Research Institute of Medical Sciences (AFRIMS) - Bangkok, Thailand

RV 91 - "Natural History of HIV-1 Infection in Thailand" -

Protocol Description:

This is a study to define the natural course of disease in Thais with HIV infection, especially those with recent infection. An understanding of early clinical, immunological and virological events will help us to establish "endpoints" for future vaccine trials. Specific objectives are:

- a. Characterize viral, immune regulatory and clinical sequelae in recently HIV-1 infected Thai men, especially during the first 1-2 years post-infection. These data may form the basis of efficacy endpoints in future prophylactic vaccine trials in Thailand.
- b. Characterize (genetically and serological) circulating HIV-1 from recently infected Thais. These data may form the basis for selection of vaccine strain prototypes for use in development of Thai-specific vaccine constructs.
- c. Assess virus specific and immune regulatory correlates of HIV-1 progression in Thais. These data will be considered largely hypothesis-generating.

Technical Approach:

Incident cases are enrolled as soon as possible after identification of seroconversion. Volunteers are bled at two time points, 6 months apart. Prevalent and seronegative controls are bled one time. Laboratory/Clinical evaluations include a Physical Exam, Skin test, FACS_{an}, PCR, CBC, and Lymphoproliferation studies.

Significant Findings:

This protocol has provided specimens for many studies and investigations done both in Rockville and in Bangkok, including dual infection and lymphoproliferative response. Analyses to address the main objectives of this study are in progress.

Incident Cases:

A small percentage of incident cases have returned for 2 visits, therefore, evaluation of decline of CD4 counts over time has not been done.

Cross-sectional determination of CD4 counts by time since estimated seroconversion:

Months	N.	Mean CD4 count	Mean CD4%
<3	2	270	17
3-5	11	661	29
6-8	9	468	27
9-11	20	462	26
12-14	10	463	20
15-17	22	462	34
>18	5	286	17

Mean CD4 for normals 813

Prevalent Cases:

Dual Infection

Positive identification of several individuals who have been infected with two different classes of HIV-1 has been completed. These results have been submitted for publication. A more detailed description of this work is contained under RV91A.

Proliferation

Lymphoproliferation assays to standard mitogens and common microbial antigens are being evaluated. Responsiveness to candida and PPD has been found to be inversely correlated to CD4 count. Efforts to measure responsiveness to HIV-1 antigens has been hampered by the lack of the purified antigens, although there is some suggestion of envelope specific responses in HIV-1 infected patients with CD4 counts >400/cu mm³.

FACSCount study

A study comparing CD4 counts determined by the Becton Dickinson FACSCount™ and FACScan™ will be conducted under this protocol using blood collected from prevalent cases. This study is slated to start in November 1994.

Assay Development

In order to validate a serologic assay for the assignment of HIV-1 subtype designations, a panel of Thai specimens is being used to cross-validate four different methods of subtype determination. Methods being tested are: subtype-B and -E specific peptide ELISAs, subtype-specific primer binding, followed by PCR, quantitative PCR using subtype specific probes, and heteroduplex mobility shift. Each technique will have certain specificity and sensitivity as well as logistic and operations considerations, but it is hoped that the parameters which make the serologic technique reliable will be defined. The performance of the assay on serum collected throughout Thailand and containing different levels of antibody will then be assessed.

Modifications:

An addendum was submitted for this protocol in August 1994 to allow additional blood draws from prevalent and seronegative participants.

Patient Enrollment:

Incident	115
Prevalent	290
Seronegative	31

Number of patients to be enrolled:

Incident	up to 300
Prevalent	up to 1000
Seronegative	up to 100

Site: Armed Forces Research Institute of Medical Sciences (AFRIMS) - Bangkok, Thailand

The following protocols are in the review process and have met initial programmatic approval. Each protocol will be described below:

RV99 - "A Phase I Trial of Biocine HIV SF2 gp120/MF59 Vaccine in Seronegative Thais" -

Protocol Description:

This trial will test a US-based (Type B) vaccine in Thais for safety and the ability to produce an immune response. It has already been tested in over 600 American subjects and is safe. It produces significant immune responses in vaccinees and has protected Chimpanzees from HIV virus challenge. The projected start date for this trial is early 1995. The vaccine product is slated to complete pre-clinical development during the next 12 months. This may be the first opportunity to test a vaccine based on the prevalent HIV-1 virus in Thailand. Specific objectives are:

- a. Determine the safety and immunogenicity of the BIOCINE HIV SF2 gp120/MG59 vaccine.
- b. Compare two different schedules of vaccination (0,1,6 months and 0,1,4 months).

Technical Approach:

This will be a randomized, controlled, clinical trial. Subjects will be bled 4 weeks after each vaccination and tested for antibody by ELISA and neutralization assays. Cellular immune responses will also be determined at selected time points.

Number of patients to be enrolled: 50

Sites: Armed Forces Research Institute of Medical Sciences (AFRIMS) - Bangkok, Thailand
Chiang Mai University, Research Institute for Health Sciences, Chiang Mai, Thailand

RV101-" Screening and Clinical Evaluation of Potential Volunteers for HIV-1
Vaccine Evaluation Trials"-

Protocol Description:

Evaluate potential volunteers for suitability for participation in clinical safety and immunogenicity trials of candidate vaccine for the prevention of HIV-1 in Thailand.

Technical Approach:

Evaluation will include collection of demographic information, medical history, laboratory evaluation (including CBC, chemistries, pregnancy test, RPR) and a psychological assessment.

Significant Findings:

Not applicable

Number of Patients to be enrolled: 20-300

Site: Thailand

RV102 - "Prevalence and Incidence of HIV Among RTA Recruits at Prachuap
Khiri Khan "-

Description of Protocol:

This protocol proposes to study the prevalence and incidence of HIV-1 infection in recruits stationed at a military post near Hua Hin in south-central Thailand. The Projected start date for this study is late 1994. Specific objectives are:

- a. Study the prevalence and incidence of HIV-1 infection in recruits stationed at two army bases in Prachuap Khiri Khan province in the Central region of Thailand.
- b. Study the feasibility of this cohort for future vaccine trials by studying attitudes and follow-up patterns.

Technical Approach:

1. Administer questionnaires and collect HIV-1 test results biannually to recruits serving with the Royal Thai Army in Prachuap Khiri Khan province.
2. Organize and implement an expanded educational and behavioral intervention program.

Significant Findings:

Not applicable, protocol still in review process.

Number of patients to be enrolled: 5,000

Site: Prachuap Khiri Khan province

RV103 - "Prevalence of HIV-1 Infections Among Recruits in the Royal Thai Army"-

Protocol Description:

This is a study to provide ongoing nationwide surveillance information about the epidemic among recruits serving with the Royal Thai Army in Thailand. The protocol will assess temporal, geographic and demographic correlates of HIV-1 infection among young men entering service with the Royal Thai Army (RTA) nationwide.

Technical Approach:

Collect demographic information on young men entering service with the Royal Thai Army (RTA) nationwide. Merge the data with routine serologic HIV data collected by the RTA.

Significant Findings: This protocol is currently in the protocol approval process.

Number of patients to be enrolled: 180,000-210,000

Site: Thailand

RV104- "Feasibility study: Vaccine Cohort Development: Adolescents: Phayao"-

Protocol Description:

A community based cohort of adolescents in the Phayao Province will be studied for feasibility for participation in future trials of HIV Vaccine(s) to prevent HIV-1 infection and disease. Collaboration has been established with Mahidol University, the MOPH Division of AIDS, and with the Provincial Chief Medical Officers for these studies. Baseline, community surveys in Phayao should be implemented by the end of September 1994. The projected start of prevalence and incidence studies is early 1995. Specific objectives are:

- a. Determine the baseline HIV-1 prevalence and HIV-1 incidence in persons attending STD clinics and anonymous test sites.
- b. Determine follow-up during a one year study period.
- c. Determine attitudes toward participating in phase III vaccine trials.
- d. Describe behavioral changes during the study period.

Technical Approach:

Subjects will be enrolled from STD clinics and anonymous test sites in and around Bangkok. Participants will be followed at 4 month intervals for one year. Education and counseling will be provided.

Significant Findings:

This protocol is currently in the protocol review process.

Number of volunteers to be enrolled: 500-1000

Site: Phayao Province

RV105- "Feasibility study: Vaccine Cohort Development: Factory Workers:
Lamphang"-

Protocol Description:

A community based cohort of adolescents in the Lamphang Province will be studied for feasibility for participation in future preventive HIV trials. Collaboration has been established with Mahidol University, the MOPH Division of AIDS, and with the Provincial Chief Medical Officers for these studies. Baseline, community surveys in Lamphang should be implemented by the end of September 1994. The projected start of prevalence and incidence studies is early 1995. Specific objectives are:

- a. Determine the baseline HIV-1 prevalence and HIV-1 incidence in factory workers in a province in northern Thailand.
- b. Determine follow-up during a one year study period.
- c. Determine attitudes toward participating in phase III vaccine trials.
- d. Describe behavioral changes during the study period.

Technical Approach:

This is a prospective, closed cohort study. Subjects will be enrolled from districts where factory workers live in Lamphang. Participants will be followed at 4 month intervals for one year. Education and counseling will be provided.

Significant Findings:

This protocol is currently in the protocol review process.

Number of Patients to be enrolled: 500-1000

Site: Lamphang Province

RV106- "Feasibility Study: Vaccine Cohort Development: Anonymous Test Sites: Bangkok"-

Protocol Description:

A community based cohort of individuals visiting Anonymous HIV test sites will be studied for feasibility for participation in future trials of HIV Vaccine(s) to prevent HIV-1 infection and disease. Collaboration has been established with Mahidol University, the MOPH Division of AIDS, and with the Provincial Chief Medical Officers for these studies. The projected start of prevalence and incidence studies is early 1995. Specific objectives are:

- a. Determine the baseline HIV-1 prevalence and HIV-1 incidence in adolescents and young adults in a province in northern Thailand.
- b. Determine follow-up during a one year study period.
- c. Determine attitudes toward participating in phase III vaccine trials.
- d. Describe behavioral changes during the study period.

Technical Approach:

This will be a prospective, closed cohort study of prevalence and incidence in individuals visiting anonymous test sites. Participants will be followed at 4 month intervals for one year. Education and counseling will be provided.

Number of volunteers to be enrolled: 500-1000

Significant Findings:

This protocol is currently in the protocol approval and review process.

Site: Anonymous test sites in Bangkok.

IV. CONCLUDING REMARKS

The Preventive Vaccines Program Area has established itself as a leader in the national and international effort in the search of a Vaccine that will prevent HIV infection. Through the initiation and execution of carefully planned basic laboratory projects, animal use protocols and human use protocols, this program area has taken an efficient and logical approach to achieving its highly ambitious goal. Through the pursuit of this goal, science has emerged which has been of significant benefit to the scientific community. Selected accomplishments include:

- Partial adaptation of an HIV-1 strain to pig-tailed macaques.
- Established dual infection with HIV-1 of genotypes B and E sets limits of breadth of antiviral immunity.
- Established concurrence of neutralization serotype with genotype regardless of geographic origin, stage of infection for genotypes B, E.
- Established inability of seronegative vaccinee sera to neutralize primary virus isolates.
- Established the intercontinental spread of HIV-1 genotype E by military populations.
- Established PCR capability for amplification of nearly full-length HIV-1 genomes.
- Completed most thorough HIV-1 incidence/prevalence study ever conducted internationally.
- Continued the only ongoing, comprehensive natural history study of a non-B genotype HIV-1 strain.
- Established a partnership for efficacy testing of subunit vaccines of genotypes E and B in Thailand.

With a functional research infrastructure in place both at the Rockville Retroviral laboratories and in Thailand, and the necessary basic and applied scientific "blueprint" established, this program area is now uniquely positioned to execute Phase I, II and III Vaccine trials. It is anticipated that the work accomplished within this Program Area, in close collaboration with other Program Areas within the HIV Research Program will directly and positively impact on the course of preventive HIV vaccine trials.

V. PUBLICATIONS AND PRESENTATIONS

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BEHAVIORAL PREVENTION

USAMRMC Program Area Coordinator:

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Program Area Summary:

The primary focus of this program area is to develop and field behavioral interventions designed to reduce exposure to and transmission of HIV infection in military medical beneficiaries. The secondary focus of the research is to provide behavioral data in support of the successful completion of preventive vaccine trials and other clinical trials.

Human Use protocols

Protocol Number	Protocol Title (Abbreviated)	Principal Investigator
RV26(C)	Tri-Service Biopsychosocial Study	Nannis
RV43A	Behavioral Addendum: AZT Resistance Study	Nannis
RV56	STD Patterns at Ft. Bragg	McKee
RV62(C)	Neurobehavioral Effects in HIV+ Aviators	Mapou
RV72	Interactive Media- Behavioral Intervention HIV Seropositive Cohort	R. Jenkins
RV76	Using Interactive Video to Reduce Risk of HIV Exposure in USAF Trainees	Zachary
RV79A	Behavioral Addendum: Multi-drug Regimen	Nannis
RV81	Behavioral Intervention - Prevention of Exposure of High Risk Soldiers to HIV/STDs	P. Jenkins
RV82(P)	Phase I/II Safety, Efficacy of Behavioral Interventions in Seropositives	Nannis
RV89	OB/GYN Affective Reactions to Uncertainty about Discussing HIV/AIDS	Goldschmidt
RV96(P)	Assessment and Modification of STD risk Relevant Behaviors - San Diego	Schneider
RV97	Functional Status Measurement to Predict Patient Compliance with Drug Regimen	Mayers
(P) Pending approval		
(C) = Closed to enrollment; data analysis phase		

I. OVERVIEW

The Behavioral Prevention Program Area has been actively involved in its current mission since July 1990. The primary focus of this research area has been to develop and field behavioral interventions designed to reduce exposure to and transmission of HIV infection in military medical beneficiaries. The secondary focus of the research has been to provide behavioral data in support of the successful completion of preventive vaccine trials and other clinical trials. This includes information that will enhance adherence to trial guidelines, willingness to participate in vaccine trials, and psychosocial factors that may influence a participant's behavior during a trial.

Germane to the primary focus there has been a major transition this year from the collection of descriptive behavioral data which created the foundation for behavioral interventions (through the Army Wide AIDS/HIV Survey--AWAS-- and the Seropositive Behavioral Survey--SBS) to the initiation of behavioral intervention protocols and development of field sites at which to test the interventions. Five protocols have been written which will assess at least 7 different behavioral interventions designed to reduce HIV transmission. These will be described in the technical approach.

There are several basic principles that underlie the Behavioral Prevention Program Area's approach to its mission. Most basic is that behavior, particularly sexual behavior, is influenced by evolutionary, genetic, and hormonal factors as well as by learned social and cultural patterns. Because of these multi-faceted influences, changes in sexual behavior are difficult to achieve through behavioral intervention and one must also assume that interventions will be differentially effective because of individual differences in these factors. In addition, demographic factors such as age, race/ethnicity, and gender and neurobehavioral factors such as coping and cognitive style will influence the impact of any behavioral intervention. Hence, one of the goals of these interventions is to identify individuals or clusters of individuals as defined by the above factors who are most amenable to the different interventions being tested.

The second basic principle underlying the Behavioral Prevention Program Area is that interventions must be developed based on extensive knowledge about the population for whom the intervention is designed. For that reason, the Army Wide AIDS Survey (AWAS) and Seropositive Behavioral Survey (SBS) data provide necessary descriptive information for developing Behavioral Prevention Program Area interventions. Major civilian research endeavors now have recognized the need for this type of descriptive information. This Program Area is unique in its foresight to incorporate this step in its initial efforts to develop research interventions. In meeting its secondary mission of providing behavioral data in support of vaccine and chemotherapy clinical trials, 3 protocols (or addenda) have been written and one is in progress. These also will be detailed in the Technical Approach.

II. RESEARCH GOALS AND OBJECTIVES

In support of the HIV Research Program's primary mission, reduction of HIV incidence among military medical beneficiaries, the Behavioral Prevention Program Area has the following goals and objectives:

- A. Develop research intervention protocols for each of the three services to reduce HIV infections in individuals at high risk for HIV infection.
- B. Develop a research intervention protocol to reduce transmission of HIV by military medical beneficiaries already infected with HIV.
- C. Provide support to the Preventive Medicine Departments of the three services through the timely dissemination of research findings, and the development of scientific strategies to evaluate existing operational HIV prevention efforts.
- D. Support the successful completion of vaccine and drug trials through behavioral research designed to understand factors that predict willingness to participate in trials, adherence with trials, and additional behavioral data that can be used to help interpret safety and efficacy issues related to trials carried out as part of the efforts of the Preventive Vaccines Program Area and the HIV Disease Prevention Program Area.

III. TECHNICAL APPROACH

The Behavioral Prevention Program Area achieves its goals through a series of research protocols and the development of an infrastructure that will allow the testing of the interventions in 4 field sites:

1. DC Metropolitan Area (Walter Reed Army Medical Center [WRAMC] and National Naval Medical Center [NNMC]),
2. San Diego, CA (HIV Neurobehavioral Research Center [HNRC] and Naval Health Research Center [NHRC]),
3. Fayetteville, NC (Fort Bragg) and
4. San Antonio, TX (Lackland Air Force Base).

The initial databases utilized for these efforts were the AWAS and SBS, both of which have been detailed in previous reports. Subsequent protocols for HIV-uninfected individuals at Navy and Air Force sites were designed with a brief, data gathering component. Based on analysis of AWAS and SBS data, the researchers were able to narrow the range of questions to those behavioral and associated risk factors that had a sound psychometric basis. This allowed for the development of a more abbreviated assessment measure for non-Army, uninfected populations.

This Program Area has created a core set of evaluation questions that can be used as part of an outcome measure for its interventions. These are questions that appear to be predictive of risk. Questions are identical in terms of wording and response format with one set of core questions to be used for seronegative intervention studies and a different set for seropositive studies. This strategy will allow for comparability of some key issues across protocols, although comparisons will need to be done at a theoretical rather than statistical level due to differences in the samples and study methodologies.

What follows is a detailed description of Behavioral Prevention Program Area protocols executed during this year. Protocols pending approvals are also described.

"Army-Wide HIV/AIDS Survey (AWAS)"

The AWAS was a representative survey of US. Army personnel conducted between October 1991 and February 1992. Previous reports have detailed the development, sampling procedures, data collection, and validation procedures of the AWAS questionnaire. These will not be discussed in the present report. Work on the AWAS during this reporting year has focused primarily on

organizing the AWAS database and ensuring quality of the inputted data. Preliminary analyses for the first major paper were performed. Eight behavioral and 3 biological factors that place an individual at risk for exposure to HIV were derived from the data. Prevalence based on a weighted percent of individuals reporting the behavior for each of the 11 risk factors (8 partner related and 3 biological) can be summarized as having: At least one "one night stand" (35.3%), Five or more sexual partners (23.0%), At least one partner of same gender (if male)* (9.1%), At least one anonymous partner¹ (13.8%), At least one prostitute as a sexual partner¹ (8.7%), At least one IDU as sexual partner² (4.4%), At least one partner with HIV/AIDS¹ (1.1%), At least one occasion of sharing needles (1.0%).

In analyzing the risk factors by age, gender, ethnicity/race, the most striking finding was that HIV exposure risk is greatest for our youngest soldiers (age 17-24). Men in the three youngest age brackets reported a statistically significant higher percentage of partner related exposure risk factors than did women: one-night stands, prostitutes, anonymous partners, sex with a man who had sex with another man, and 5 or more sex partners. In the case of women in the youngest age bracket, they were statistically more likely to report at least one STD during the past 2 years and to report having at least one partner with genital/anal sores. This finding remained even when marital status was considered.

When gender is considered in the context of race/ethnicity, the two factors interact when reporting at least one STD. African-American women had the highest percentage reporting a STD (10.8%); almost twice as many African-American women as African-American men reported having a STD over the past 2 years. White, non-Latina women reported a STD significantly more often than did white men, but only 1.2 times more. These findings concerning increased report of STDs by women, and in particular African American women, has spurred the Behavioral Prevention Program Area to begin to develop a protocol focusing on women's HIV/STD exposure risk, particularly, women of racial/ethnic minorities.

Additional analyses from the AWAS are in progress.

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1. The recall period for these risk factors is 12 months prior to the survey.
 2. The recall period is two years prior to the survey.

* if female, male partner had sex with men ²

RV26 - "Tri-Service HIV Biopsychosocial Study" -

Overview:

The purpose of the Tri-Service HIV Biopsychosocial Study was to provide descriptive information about HIV transmission risk and related factors in HIV-infected individuals in the military. This information was to be used as the foundation for developing behavioral interventions to reduce HIV transmission.

Protocol Description:

The major objectives of the study were:

- a. Identify specific behaviors and associated factors that relate to HIV transmission risk behaviors in HIV-infected military medical beneficiaries.
- b. Provide descriptive information about psychosocial functioning and coping styles in HIV-infected military medical beneficiaries.
- c. Provide descriptive information about neuropsychological functioning over time and relate this to job functioning.

Technical Approach:

The study contained four distinct components: the anonymously administered Seropositive Behavior Survey (SBS), the Psychosocial Questionnaires (PSQ), the Structured Clinical Interview for DSM-III-R (SCID), and the Neuropsychological Assessment. All have been described in previous reports. During the past year, data collection for the neuropsychology and psychosocial portions were conducted. Data collection for all portions of RV 26 ended on 1 June 1994. Further efforts on this protocol will be in the area of data analyses and report of findings.

Psychosocial Questionnaires (PSQs)

Technical Approach:

Psychosocial data was collected from military medical beneficiaries (MMBs) for the purposes of documenting levels and manifestations of psychological dysfunction that may occur with HIV-infected individuals and interfere with their social/occupational functioning. Data collection involved the use of self-report, standardized questionnaires.

The core questionnaire measures, which were administered throughout RV26 encompassed a number of domains. These included: depression (Beck Depression Inventory; Beck & Steer, 1987), anxiety (Spielberger State Anxiety Inventory; Spielberger, 1983), transient mood states (Profile of Mood States; McNair, Lorr, Droppleman, 1981), coping styles (Cortauld Emotional Control scale [Watson & Greer, 1983]; Multidimensional Health Locus of Control scale [Wallston, Wallston, & DeVellis, 1978]; Temoshok Coping Vignettes), and social functioning (short-form version of the UCLA Loneliness scale; Hays & DiMatteo, 1987). Most of these measures are being used in civilian studies of HIV-infected individuals, providing comparability with the Multicenter AIDS Cohort Study (MACS) and other important cohorts. They also have been used widely with other populations, including those with medical illnesses, lending comparability to other disorders and life circumstances. Standardized questionnaires such as these have the advantage of being easy to administer and providing reliable and valid information.

A total of 1233 HIV-infected military medical beneficiaries (MMBs) completed the questionnaires at Time 1. Those completing Time 2 questionnaires number 788; Time 3 = 428 respondents; Time 4 = 195 respondents; Time 5 = 77 respondents. Time 6 = 24 respondents; Time 7 = 4 respondents. Several questionnaires were originally included in this battery of instruments but were discontinued because they were empirically redundant with other measures (e.g., Symptom Checklist-90 Revised [Derogates, 1979]; Schaefer, Coyne, & Lazarus (1981) Social Support Inventory; Zich & Temoshok Social Support scale [Zich & Temoshok, 1987] and Spielberger Trait Anxiety Inventory [Spielberger, 1983] or unrelated to important psychosocial outcomes (Kobasa Hardiness scale [Kobasa, Maddi, & Kahn, 1982]). Each of these measures were used in the early phases of the study; they were completed by 400-700 individuals, and depending on the measure, typically completed only at Time 1. Several questionnaires were added to the study to assess specific issues that appeared important to our population. These included the Perceptions of AZT questionnaire (Pivar & Temoshok, 1989) and a religious coping questionnaire (Pargament, Grevengoed, Hathaway, Kennel, Newman, & Jones, 1988). These later measures have been completed by 850 individuals at Time 1, 324 at Time 2, 77 at Time 3, and 8 at Time 4. Most recently, a questionnaire was added to investigate attitudes and beliefs relevant to participation in vaccine and drug therapy trials. This measure has been adapted from the AZT questionnaire.

Selected Findings:

Analysis of psychosocial data has focused on two areas: work performance and a description of psychosocial factors in women. Comparing 40 HIV-infected military personnel with a group of HIV-uninfected military personnel matched on gender, race/ethnicity, age education level, and rank on a series of self report questionnaires, the researchers found that HIV-infected

individuals perceived themselves as less productive, experiencing more on the job difficulties, and have fewer work demands than the HIV-uninfected group.

A second set of analyses explored the differences between HIV-infected women and an equated group of HIV-infected men on psychosocial and behavioral variables. As compared to the men, women reported that they used and were satisfied with their level of social support. There also was a trend towards significance with women reporting greater levels of anxiety than men. With regard to transmission behavior, women were less likely to have new sexual partners or go out looking for new partners during the six months; when they had sex during the six months it was more likely to be with their steady partner or spouse than with any other types of partners. Women consumed less alcohol than men and reported less general risk taking.

Neuropsychological Component

Technical Approach:

Subjects were assessed at approximately six month intervals. A combination of standard and experimental neuropsychological measures was selected based on prior research at military sites to 1) provide assessment of all realms of cognitive and motor function, and 2) assess in depth those areas most likely to be affected at the early stages of HIV disease. Two alternating batteries were used to maximize the number of measures administered while not increasing evaluation time further.

Battery A, requiring 3 hours, included measures of estimated intellectual level (American version of the Nelson Adult Reading Test [Grober & Sliwinski, 1991]; Vocabulary and Block Design subtests of the Wechsler Adult Intelligence Scale-Revised [WAIS-R, Wechsler, 1981]), attention and speed of information processing (Digit Span and Digit Symbol subtests of the WAIS-R) [Wechsler, 1981]; Visual Memory Span subtest of the Wechsler Memory Scale-Revised [Wechsler, 1987]; Trail Making Test [Reitan & Wolfson, 1985]; Digit Vigilance [Heaton, Grant, & Matthews, 1991]; Paced Auditory Serial Addition Test [PASAT; Gronwall, 1977]; Simple and Choice Reaction Time tasks [Martin, et al., 1992]), motor functions (Strength of Grip [Reitan & Wolfson, 1985]; Grooved Pegboard Test [Kløve, 1963]), language skills (Boston Naming Test [Kaplan, Goodglass, & Weintraub, 1983]; Controlled Oral Word Association Test [Lezak, 1983], Vocabulary subtest of the WAIS-R [Wechsler, 1981]), visuospatial skills (Block Design subtest of the WAIS-R [Wechsler, 1981]; Standardized Road Map Test of Direction Sense [Money, 1976]), learning and memory (Digit and Visual Memory Supraspan procedures [Benton, Hamsher, Varney, & Spreen, 1983]; Story Memory Test [Heaton, Grant, & Matthews, 1991]; Figure Memory Test [Heaton, Grant, & Matthews, 1991]; Rotary Pursuit [Heindel, Butters, & Salmon, 1988]), and problem-solving (Category Test [Reitan & Wolfson, 1985]).

Battery B, requiring 2.5 hours, included measures of attention and speed of information processing (Arithmetic subtest of the WAIS-R [Wechsler, 1981]; PASAT [Gronwall, 1977]; Simple and Choice Reaction Time tasks [Martin, et al., 1992]; Posner Spatial Cueing Task [Posner, Cohen, & Rafal, 1982]; Sternberg Memory Search Task [Sternberg, 1966]), motor functions (Finger Tapping Test [Reitan & Wolfson, 1985]; Grooved Pegboard Test [Kløve, 1963]) and learning and memory (California Verbal Learning Test [Delis, Kramer, Kaplan, & Ober, 1987]; Rey-Osterrieth Complex Figure Test [Lezak, 1983]).

Computerized Cognitive Tasks:

A subset of tasks from COGSCREEN (Kay, 1994), a group of computerized cognitive tasks which evaluate accuracy and reaction time in several different cognitive realms, were added to Battery B in November 1991, at Washington, DC sites. Included were measures of attention (Visual Sequence Comparison; Divided Attention Task), spatial awareness (Mannikin Task), and short term learning and memory (Delayed Matching to Sample Task).

Self-Report Performance Measures:

Standardized questionnaire measures of self-reported cognitive and motor functioning (Awareness of Functioning, Downer et al., 1991), job performance (Employment Questionnaire, Downer et al., 1991), and job satisfaction (Job Descriptive Index, Balzer & Smith, 1990), were added to Battery B at Washington, DC sites. A subset of items from the Awareness of Functioning questionnaire also was administered to subjects at WHMC, beginning in November 1991.

Significant Findings:

Reaction time (RT) performance was examined in more detail to understand underlying impairment and to develop measures sensitive to cognitive changes in the early stages of disease. A series of studies found that 1) HIV-infected subjects did not show a deficit in working memory relative to HIV-uninfected control subjects, although, they were significantly slower on several RT measures; and 2) HIV-infected subjects were found to be slower than HIV-uninfected subjects on all RT measures, replicating prior findings. In addition, although HIV-uninfected subjects improved their performance when given extra warning time before having to respond, HIV-infected subjects showed no such improvement; 3) HIV-infected subjects were significantly slower on all conventional tasks and on 3 of 6 computerized tasks measuring reaction time.

Number of Patients Enrolled: 1418 seropositive
152 seronegative control subjects

Total Patient Visits: 404

Sites for RV26: Walter Reed Army Medical Center, Washington, DC
National Naval Medical Center, Bethesda, MD
Wilford Hall Air Force Medical Center, San Antonio, TX

RV26 Addendum- "Neurobehavioral Changes In Early Human
Immunodeficiency Virus Infection: Foundations for Study of Military
Performance"-

Protocol Description:

The purpose of the addendum was to build a foundation for the measurement of possible HIV-related changes in military performance. Specific objectives included:

- a. Demonstrate that selected computerized cognitive measures of performance were sensitive to neurobehavioral changes associated with HIV.
- b. Link performance on these measures to self-reported job performance and satisfaction.

Technical Approach:

A subset of subjects from the main study was selected and recruited for participation. One group of individuals was selected on the basis of having participated in an earlier neurobehavioral study at Walter Reed Army Medical Center (WRAMC), in order to understand the longer term effects of HIV. A second group of individuals was selected on the basis of having no pre-existing neurobehavioral disorder, in order to understand more clearly the effects of HIV alone. Subjects were evaluated every 6 months in conjunction with regular RV26 visits. In addition to RV26 measures, subjects completed a brief neurological screening examination, MRI scan, lumbar puncture, computerized cognitive measures, and self-report measures of occupational performance and satisfaction. Neurodiagnostic measures were included to determine whether non-specific neurological abnormalities due to HIV have any bearing on behavioral measures of performance and whether these neurological abnormalities should be used as the basis for job reclassification decisions. Data collection was completed on 30 June 1993, as reported previously. The study is in the data analysis phase.

Significant Findings:

Data from initial evaluations of subjects who completed lumbar puncture (N=20) were analyzed. Subjects were grouped by whether cerebrospinal fluid culture was positive (CSF+ infected, N=9) or negative (CSF- uninfected, N=11).

CSF+ infected subjects had significantly higher levels of quinolinic acid, an endogenous neurotoxin previously shown to be increased in HIV-infected individuals, significantly higher CSF white blood cell counts, and significantly slower reaction times than CSF- uninfected subjects. Results suggested a relation between biological markers of central nervous system infection (CSF HIV culture, quinolinic acid, white blood cell count) and a behavioral measure of cognitive change.

Number of Patients Enrolled: 37 HIV-infected subjects

Patient Visits: Not applicable

Site: Walter Reed Army Medical Center, Washington, DC

RV56-"Analysis of Sexually Transmitted Disease (STD) Patterns at Ft. Bragg, NC:
Preparation for Human Immunodeficiency Virus Behavioral Interventions"-

Protocol Description:

This protocol was designed as an epidemiological surveillance and analysis protocol, the purpose of which is to provide insights into patterns and trends of sexually transmitted diseases at the Army's largest fixed facility. Data is analyzed to provide an understanding of STD activity at Fort Bragg, which can be used in support of behavioral intervention protocols, and to identify targets for behavioral and biological interventions designed to reduce risks of acquiring HIV and other STDs. The objectives of the study are as follows:

- a. Determine the incidence of specific STDs at Fort Bragg.
- b. Describe epidemiological characteristics of individuals who acquire STDs.
- c. Identify demographically determined groups which would be targets of sexual history questionnaires and behavior interventions in future protocols.

Technical Approach:

The STD Clinic at Fort Bragg (EDC Clinic) provides centralized service for all military health care beneficiaries at Fort Bragg. All individuals presenting with signs/symptoms and/or positive laboratory findings for sexually transmitted diseases are managed through this clinic. A centralized database for all individuals seen through the clinic has been maintained since 1984. This

database contains comprehensive demographic, clinical, laboratory, diagnostic, and therapeutic information for each patient and patient visit.

The EDC Clinic and this database, as utilized in RV56, provide a framework for intervention studies to reduce HIV risk-relevant behaviors in the at-risk populations at Fort Bragg. This information will be used to provide the epidemiological basis for behavioral and biological interventions designed to reduce the risk of acquiring STDs.

An Addendum to RV56, designed to provide an enhanced diagnostic laboratory capability to improve the quality of data collected under RV56 and other Behavioral Prevention Program Area protocols, was prepared and concept-approved by the Retrovirus Clinical Review Committee in February 1994. This addendum outlines a strategy for collaboration with the STD Center at the University of North Carolina at Chapel Hill to provide intellectual and operational assistance in the general design and development of HIV and STD prevention and control measures, focusing on the use of STDs as objective outcome markers for studies to reduce HIV risk-relevant behaviors. The initial focus of this addendum was the development of a plan to provide state-of-the-art diagnostic clinical microbiology support for RV81. A contract has been finalized between the Foundation and the UNC/Chapel Hill to 1) design all necessary protocols for the collection of specimens; 2) to provide specific microbiological/immunological tests for diagnosis of certain STDs; 3) to provide training for Fort Bragg personnel involved in specimen collection; and 4) to prepare an implementation plan for coordination of all efforts surrounding specimen collection and transport from WAMC to UNC/Chapel Hill.

Significant Findings:

No formal analyses of STD trends and patterns were conducted during the 1-year reporting period. However, information contained in the database was examined for assistance in design of the proposed Women's Study ("Needs assessment for developing targeted behavioral interventions for female and minority soldiers at high risk for STD/HIV infection; Principal Investigator: Pamela R. Jenkins, MSN) and local extractions have been made to provide monthly input since July 1994 for the Command Health Report prepared for the Womack Army Medical Center (WAMC) hospital commander.

Number of Patients Enrolled: Not applicable; this is a surveillance protocol.
Numbers of patients studied equates to number of visits, below.

Patient Visits: 11,567 (Total EDC Clinic Visits)

Site: Fort Bragg, NC

RV62-"Neurobehavioral Effects of Early Human Immunodeficiency Virus Infection: Foundations for Study of Military Aviator Performance"-

Protocol Description:

The purpose of this protocol was to determine how HIV may affect aviation performance. The objectives of the study were:

- a. Determine the prevalence of neurobehavioral deficits in HIV infected aviation personnel and progression of deficits over time.
- b. Clarify relations among neuropsychological changes, mood state, job satisfaction, and perceived changes in job performance.
- c. Establish a core battery of measures sensitive to early changes in performance, which may be applied to clinical screening of aviators.
- d. Establish initial targets of intervention to minimize the effects of disease on performance and to maximize occupational longevity.

Technical Approach:

The Principal Investigator planned to study at least 15 HIV-infected aviation personnel, including pilots, navigators, radar control operators, weapons systems controllers, and air traffic controllers, for 3 years, and at least 15 demographically-matched HIV-uninfected control subjects for one year. Subjects were to be evaluated every 6 months. Subjects completed neurological examination, neurodiagnostic evaluation (ERP, SPECT), neuropsychological evaluation, computerized cognitive assessment, and standardized, validated self-report questionnaire measures of depression, anxiety, job performance, job satisfaction, and awareness of changes in function. This was a comprehensive evaluation, designed to detect abnormalities most likely to impact on performance, particularly changes in attention, response speed, motor skills, visuospatial skills, and learning/memory. Neurodiagnostic measures were included to determine whether non-specific neurological abnormalities due to HIV have any bearing on behavioral measures of performance and whether these neurological abnormalities should be used as the basis for job reclassification decisions. Yearly evaluations included all measures. Intervening 6 month evaluations included ERP and a subset of neuropsychological measures. Enrollment and data collection were terminated on 30 April 1994 due to 1) complicated pre-existing neuropsychiatric history in most participants that would limit ability to attribute findings to the effects of HIV alone, and 2) shifts in program priorities.

Significant Findings:

6 HIV-infected subjects completed baseline evaluation, and 1 HIV-infected subject completed follow-up evaluation.

Number of Patients Enrolled: 8 HIV-infected subjects; 1 HIV-uninfected control subject

Site: National Naval Medical Center, Bethesda, MD

RV72-"Using Interactive Media to Promote Responsible Sexual Behavior in HIV Positive Personnel"-

Protocol Description:

This study is an evaluation of an interactive video program to reduce HIV transmission risk-relevant behaviors in HIV-infected military personnel.

The objectives of this study are:

- a. Examine the use of an interactive video disc (IAVD) intervention to reduce the risk of exposure to human immunodeficiency virus (HIV) and other sexually transmitted diseases (STDs) in a general military population.
- b. Evaluate the safety of video material, in terms of its ability to transmit accurate HIV behavior change messages in a highly motivating manner.
- c. Evaluate the immediate impact of the video in terms of changes in knowledge, attitudes, and behavioral intentions.

Technical Approach:

Educational materials are currently being used by preventive medicine personnel in HIV evaluation units to provide instruction regarding disclosure of HIV serostatus, negotiation of responsible sexual practices, and self-evaluation of current HIV transmission risks. Concerns about the accuracy of the current material used and motivational value of this program have been raised since its release. Hence, the evaluation addresses these concerns through two components: (1) a Delphi panel of HIV research and service professionals will

evaluate the accuracy of information regarding HIV transmission risk, while (2) a pre/post evaluation study with HIV-infected personnel will evaluate the video's impact on its intended audience.

The pre/post evaluation will be conducted with a sample of 60 HIV infected patients at Fort Bragg, NC. Pre-video data will include information regarding demographics, recent HIV education, and self-report information concerning HIV transmission risk-relevant knowledge, attitudes, and behavior. Post-video data will include quantitative and qualitative assessments of the video's impact including its contributions to motivations to reduce HIV transmission risk-relevant behaviors.

The protocol has received Tri-service scientific approval and approval by the Army Personnel Survey Office, Commander US Army Research Institute, Alexandria, Va. It is awaiting Tri-Service Human Use approval.

Significant Findings: Not applicable

Number of Patients Enrolled: None

Patient Visits : None

Sites: Fort Bragg, NC (Patient Component)
1 Taft Court, Rockville MD (Delphi Component)

RV76-"Use of Interactive Media to Reduce the Risk of HIV Exposure in United States Air Force Basic Trainees"-

Protocol Description:

This protocol is designed to evaluate the effectiveness of two large group interventions -- an interactive video versus a more traditional lecture -- in reducing the risk of HIV exposure in a general (seronegative) military population. Both interventions are compared against a standard (no-treatment) comparison group. Phase I (pilot) and II (main) studies differ in terms of sample size (N=536 versus 1200) and length of follow-up (5 to 6 weeks versus 4 to 5 months). The outcome measure, which is based on the most predictive items from the Army-Wide AIDS/HIV Survey, is intended to assess changes in HIV-related knowledge, attitudes, beliefs, and behaviors as well as in biological markers for various sexually transmitted diseases. The specific objectives are as follows:

- a. Estimate the incidence rates of specific high-risk behaviors in a sample of those attending military police training, and compare these rates at least qualitatively to other militarily relevant samples (e.g.; the all-Army survey, STD clinic patients).
- b. Define demographic and behavioral correlates of individuals in low-risk versus high-risk groups.
- c. Test the effectiveness of an interactive media, large group intervention in reducing HIV-relevant risk behaviors in first-term U.S. Air Force (USAF) enlisted personnel over a brief (5- to 6-week) and a more moderate (4- to 5-month) period.
- d. Determine which aspects of the intervention were effective, and develop more specific behavioral targets for future interventions.

Technical Approach:

The present protocol was conceived as an initial study to test the effectiveness of an interactive media, large group intervention in reducing HIV-relevant risk behaviors in a (seronegative) military population. The basic design is a randomized field experiment with subjects (USAF trainees), nested within training flight, randomly assigned to one of three treatment conditions (lecture, interactive video, or control). The main dependent measures are knowledge, attitude, and behavioral self-report variables assessed pre and post intervention. The prototypical analysis is a repeated-measures analysis of variance to assess the effects of treatment condition (randomized factor) and measurement occasion (nested factor) on the various dependent measures.

Phase I (pilot study) was conducted at the Joint Security Police/Law Enforcement Training Center (343rd Training Squadron) at Lackland AFB. Five hundred thirty-six subjects were administered a pre-test questionnaire covering their current attitudes, beliefs and behaviors and then given either a lecture presentation or an interactive media presentation, or no presentation (control group) at all. Six weeks later, at the completion of their tech school training, they were given the same questionnaire again and the ratings were compared to determine any behavior or attitude changes that may have occurred as a result of the interventions.

The interventions for the Phase II (main) study are scheduled to begin on 6 October 94. The study designs for Phases I and II are similar except that, in Phase II, the follow-up period is extended from 5-6 weeks to 4-5 months, and the sample size is increased to 1200 trainees. Based on pilot data, the researchers believe that the base rates for most high-risk behaviors were too low during the brief follow-up period for us to make meaningful comparisons across interventions. The follow-up period was extended to 4 - 5 months.

Significant Findings:

Preliminary results from the pilot study were generally similar to the results from the Army-wide HIV/AIDS survey. The initial questionnaire data revealed that 296/536 (55.2%) of the sampled trainee population reported ≥ 1 HIV-relevant risk behaviors in the past 12 months. Of these individuals, 147/536 (27.4%) reported ≥ 2 risk-relevant behaviors. These were all behaviors that carry a clear risk for acquiring or transmitting HIV and other sexually transmitted diseases. These risk behaviors, and their reported percentage of occurrence in the past 12 months at baseline, were: one-night stands (44.8%), ≥ 4 sexual partners (13.4%), non-menstrual bleeding (12.3%), anonymous sexual partners (8.8%), group sex (8.0%), any STDs (4.1%), gay or bisexual male partner (3.7%), receptive anal intercourse (3.7%), injectable drug user as a sexual partner (3.0%), HIV-infected sexual partner (2.4%), sex with prostitutes (1.5%), genital/anal sores (1.1%), and sharing of drug needles (0.0%). There is no question that many of these behaviors represent very high-risk situations. For example, of the 215 individuals who reported having one or more one-night stands in the past 12 months, 136/215 (63%) reported that they did not always use a condom. Moreover, these rates were not significantly different for men (64.0%) and women (59.6%).

In contrast, the follow-up behavioral data looked very different. Although individuals still reported engaging in some high-risk behaviors, the percentages were much lower. This difference could not necessarily be attributed to the interventions and may be purely artifactual. In particular, it was problematic to compare unequal time periods (4 weeks vs. 12 months). In addition, the individuals in this study were participating in an intense training session where they were living in dormitories and their extra-curricular movements were significantly restricted. Hence, any figures obtained during this training period might underestimate the more typical rates of risk behaviors that these same individuals would engage in once they arrive at their first duty session. It was for these (and other) reasons that the researchers planned to extend the follow-up period in Phase II of the study to 4-5 months. Although, again there will be unequal time periods (12 months vs. 5 months) it is believed there will be a better opportunity to sample behavior and possibly extrapolate results.

The planned Phase II study, which was scheduled to begin on 6 Oct 94, will help address this issue more directly and will provide data to assess the differential efficacy of the 3 intervention formats.

Number Of Subjects Enrolled: 536/536 (Phase I)
0/1200 (Phase II)

Site: Lackland Air Force Base, San Antonio, TX

RV81- "Prevention of Exposure to HIV and other Sexually Transmitted Diseases (STDs) in a Seronegative Military Population: A Comparative Study of the Safety and Efficacy of Intensive STD/HIV Prevention Interventions"-

Protocol Description:

This study is designed to examine the relative safety, feasibility, and effectiveness of three interventions designed to reduce exposure to human immunodeficiency virus (HIV) and other sexually transmitted diseases (STDs) in a population at high risk for HIV infection (active duty military STD patients). The objectives of the study are as follows:

- a. Develop empirically-based HIV/STD prevention interventions to motivate and maintain individual behavior change consistent with preventing exposure to HIV and other STDs.
- b. Evaluate the feasibility and safety of delivering the proposed HIV/STD intervention programs to a high-risk military population (STD patients).
- c. Evaluate the immediate impact of interventions on compliance with standard STD recommendations (e.g., return for test of cure, partner notification), as well as risk-related knowledge, attitudes, perceptions of risk, and readiness for change.
- d. Determine the efficacy of various interventions in reducing the rates of risk-relevant behaviors and related factors.
- e. Evaluate the effectiveness of the interventions in lowering STD incidence and recidivism rates.
- f. Describe demographic, attitudinal and behavioral characteristics of individuals resistant or responsive to behavior change in order to design more effective programs.

Technical Approach:

A pre/post, group comparison design will be used, in which the various interventions delivered in tandem with "standard" clinical care/counseling will be compared with each other and with the standard STD clinic care alone. Baseline data will be collected at entry into the study, while post-test data will be collected two weeks later when patients return for their normal follow-up visit. A second follow-up visit will be conducted at two months after entry into the study. This two month follow-up will be used for the purpose of evaluating the logistics of following patients over a relatively long period of time for data collection and, possibly, intervention purposes. This follow-up is not being treated as a formal part of the study because it is expected that a variety of methods for follow-up may need to be implemented and tested.

Research findings from the AWAS were translated into three distinct intervention programs. Two building on existing military resources (interactive video and health risk appraisal), while a third brings innovative approaches to the traditional modality of one-on-one patient counseling (targeted situational behavior).

The interactive video disc (IAVD) requires active involvement of viewers and has shown itself to be acceptable and, in the short run, efficacious in altering HIV risk relevant attitudes and behavioral intentions in STD patients at Ft Bragg. The IAVD material has been selected from a series of interactive videos developed for the US military for the purpose of reducing HIV risk behaviors. The "Risk" module of Body Armor was selected. The material presented in this module is congruent with AWAS findings.

In its generic form the health risk appraisal (HRA) has been used by the Army for more than a decade. Using the findings from the AWAS, a STD/HIV computer scannable Health Risk Appraisal (HRA) was developed. After the patient completes the questionnaire it is computer scored and individual feedback messages are generated for each variable or group of variables. The individualized results and recommendations are given to the patient along with counseling to enhance individual perception of risk and provide suggestions for relevant ways to change personal behavior. A contract was executed with Healthier People, Inc. to develop the software for the HRA. This is the same company that developed the Army's generic HRA. Using the results obtained from the AWAS, those questions that demonstrated significant results were grouped and individual feedback messages were developed for each group. Graphic displays, as well as individual messages will be computer generated from the scannable questionnaire for each patient.

Targeted Situational Behavior (TSB) consists of an individual behavioral intervention to address the specific risk behaviors and associated factors identified through the AWAS and focus groups conducted with STD patients at

Ft Bragg. It addresses the most frequently occurring scenarios which pose the highest level of HIV exposure risk. The individual is asked to place himself in the scenario that most matches how they meet potential partners (i.e. go to a club to drink and pick up girls). At a critical juncture in the scenario the staff member will stop the scenario, and ask the participants to identify other possible courses of action. In this manner, the individual will be assisted in assessing all options he or she may have in that situation, many of which may not have been considered before. This technique is similar to the thought-stopping techniques commonly used in behavioral interventions. It is theorized that this technique assists individuals in changing dysfunctional attitudes and motivates them to use the newly identified options.

In addition, an RV81 addendum has been submitted. This was done at the request of the scientific review committee in order to clarify some items in the protocol, and in order to expand RV81 to incorporate the collection of sophisticated biological tests from the study volunteers. Although the addendum will not change the behavioral intervention portion of this protocol in any substantial way, the addendum provides an expanded and elaborated plan to provide biological data to support behavioral outcome data. The biological assessment will be carried out through the contract with the University of North Carolina-Chapel Hill, that was described previously under the RV56 addendum. The results from these additional biological tests will be compared to the self-reported behavior measures collected as part of the study. The biological tests will be done in collaboration with University of North Carolina-Chapel Hill, through the RV56 addendum.

Significant Findings:

Results from pre-tests demonstrated that the IAVD is well received and soldiers have no problems using it. The module selected can be seen within the 20 minutes allotted for interventions. The Targeted Situational Behavior (TSB) intervention has been the focus of most of the pilot testing as this is the most interactive of all the interventions. The typical scenario developed by the research team has held to be fairly accurate of what is happening in these soldiers' lives. Additional scenarios (i.e., how to deal with a soldier who states he does everything right, or how to deal with a soldier who presents with an inconsistent story) have been developed and are currently undergoing pre-testing. This intervention also can be conducted within the 20 minutes available. Once the HRA/Baseline behavior outcome measure form is received from the printer, it also will be pilot tested to assure that the individual can complete the form, and that it can be scanned and counseling can be provided within the allotted time. There will be more time available for this intervention as the HRA is also the main behavioral outcome measure, so the individual will not need as much time at the end to complete outcome measures.

Number of Patients Enrolled: Not applicable

Patient Visits: 25 Focus Groups have been conducted
49 Individuals participated in pre-testing

Site: Fort Bragg, North Carolina is the sole site for this protocol

RV82-"Phase I/II Study of Safety, Behavior Responsiveness, and Efficacy of Behavioral Interventions in HIV Seropositive Military Medical Beneficiaries"-

Protocol Description:

This protocol is designed to assess the safety and efficacy of three research intervention formats. The primary goal of the interventions is to reduce HIV-transmission risk behaviors in seropositive military medical beneficiaries. Secondly, the interventions are designed to change related factors that ultimately will lower transmission risk behaviors (e.g., increase one's motivation to change behavior). The objectives of the study are as follows:

- a. Develop first generation intervention modalities to reduce HIV transmission risk potential in HIV seropositive military medical beneficiaries, based on findings from the Seropositive Behavior Survey component of the Tri-Service Biopsychosocial HIV Study.
- b. Evaluate the safety and behavioral responsiveness parameters of these behavioral interventions.
- c. Evaluate the efficacy of a "booster" treatment in reducing the HIV transmission risk potential of HIV seropositive military medical beneficiaries.

The effectiveness of the different formats for participants is expected to vary in relation to several characteristics: demographics, stage of infection, pre-existing coping style, and pre-existing behavioral proclivities relevant to transmission risk potential. Distinct clusters of individuals will be identified, based on these characteristics for which the different interventions or combinations of interventions will be most beneficial for reducing transmission risk-relevant behaviors. In addition, cognitive factors such as attention, planning ability, impulsivity, learning and remembering what has been learned are also thought to contribute to differential efficacy and will be measured in this study.

The development phase of this study has involved an iterative process of (a) translating research results into basic intervention content frameworks; (b) conducting focus groups with patients and military health care providers to

develop the content within these frameworks and to determine the most promising vehicles (formats) to carry the content messages; and (c) modifying content, messages, formats, and implementation procedures based on input from preventive medicine co-investigators and service/site-specific health care providers in the clinics where the interventions are intended for implementation. Further, specific care has been taken to ensure that ethnic and cultural factors are considered. The data on which the interventions are based comes from the SBS with a representative ethnic sample. The focus groups of HIV-infected individuals which have been used to drive the content and format of the interventions, as well as to provide feedback on what has been proposed have all included minority membership, particularly African-American participants.

Three types of interventions are planned: HIV Transmission Risk Assessment Profile with Individualized Counseling (HTRAPIC), Changing Risk Relevant Behavior Group, and the Changing Transmission Risk Behavior Audiotape Intervention. These interventions were chosen because they represent the most feasible format alternatives currently available, and a range of modalities for the presentation of information. They also are sufficiently different from one another to allow for meaningful comparisons across formats. Content of the interventions regarding transmission risk-relevant behaviors has been designed to be as consistent as possible across formats, and has been developed to address the major findings from the RV26-SBS.

The principle guiding the study design is that the research interventions to be tested must address similar content areas. These areas have been identified by the RV26-SBS data base as factors that are statistically significant in their relation to transmission risk relevant behaviors. In addition, each of these areas was discussed in a series of focus groups to provide support for the ecological validity of the content. These areas include: drug and alcohol use, numbers of sexual partners, safer sex negotiation skills, types of sexual partners, situations in which sexual partners are sought, types of sexual practices in which one engages, attitudes towards condoms, amount of social support, breadth of social activity repertoire, and disclosure of HIV status. By virtue of the difference in formats, the areas are dealt with in different degrees of depth or emphasis, but all areas are addressed in each intervention.

Critical to a successful intervention is enhancing participants' motivation to change. Three key elements that are hypothesized to increase motivation are: individualized content that allows a participant the opportunity to address an issue of personal relevance, a heightening of one's perception of personal risk; and an interactive/feedback loop for proposed change. The three proposed interventions differ with respect to how many of the three elements are included in the intervention technology. They range from the HIV Transmission Risk Assessment Profile with Individualized Counseling (containing all three elements) to the Changing Risk Relevant Behavior Audiotape (containing only minimal interaction).

Intervention Technologies

The HIV Transmission Risk Assessment Profile with Individualized Counseling (HTRAPIC):

The risk assessment format for this intervention is adapted from the more general Health Risk Appraisal (HRA) utilized by the Military Services. The basic principle for this was previously described in the RV81 summary. Based on analysis of RV 26-SBS data, the 10 content areas identified as statistically significantly associated with transmission risk-relevant behavior have been empirically derived. Those items from the SBS that assess these areas are used as items for the risk assessment. Based on an individual's responses, s/he will be described according to a transmission risk profile (empirically based on the SBS). Each profile "type" will have associated standard written "behavioral prescriptions" for individuals to read. These brief prescriptions then become the guide for counselors/health educators in delivering more elaborated, individualized counseling to that person.

The counseling will be conducted according to standard procedures, although the exact content will differ across individuals. Based on the participants' profile, counselors will review the computerized output to ensure that the messages are understood. The counselor will then explain the self control strategy that is a common denominator of all 3 interventions; that is: "Stop, Think, and Act Responsibly," in the context of a scenario written for this purpose. The use of these scenarios will be similar to that previously described in RV81.

Changing Risk Relevant Behavior Group (RRBG):

The RRBG intervention is structured not only to allow HIV seropositive military personnel to acquire knowledge but also to examine their attitudes and beliefs while developing alternative, safer behaviors. The intervention consists of a core curriculum that is facilitated by 2 co-facilitators. Within the context of two, 2 hour group sessions, the 10 risk factors described above are presented and addressed within the group. These two sessions, or modules, have been developed with input from potential participants through focus group sessions. Participants are asked to discuss key situations/behaviors and using both the facilitators' input and input by other group members. Participants are guided to develop alternative behaviors that have been found by the SBS to be related to lower transmission risk behavior. These modules also include planned "exercises" in which participants take part. The examples and content of the group are based on issues and information that participants bring to the group. By having participants generate the content, the group is more likely to foster a sense of individual relevance for its participants. However, the facilitator's manual includes key issues and possible situations which can be utilized in the group if critical issues are not introduced by group members.

The group format of the RRBG is not to be confused with either a therapeutic or support group. RRBG groups utilize a teaching/facilitation approach and provide learning opportunities for alternative behaviors, behavioral modeling, and the rehearsal of HIV prevention strategies aimed at preventing transmission risk-relevant behaviors. Unlike the vast preponderance of other intervention research in the literature, the proposed intervention focuses solely on seropositive individuals, is empirically based in terms of content, and the group process is one of facilitated interaction rather than instructional or didactic. In addition, unlike interventions in the civilian community, the CRRBG focuses on both the development of more appropriate behaviors and a modification of underlying psychosocial factors (e.g., anxiety) that can motivate and sustain unsafe behavior.

The Changing Risk Relevant Behavior Audiotape:

The audiotape intervention capitalizes on the importance of providing information when the individual is ready to hear it and most likely, therefore, when the individual can most benefit. The tape will be narrated by HIV-infected military medical beneficiaries who will interweave their own experiences with more general content relevant to transmission prevention. This strategy capitalizes on our preliminary finding that those with greater access to HIV infected peers tend to have lower HIV transmission potential (Blake, et al., 1992). This intervention format also should enhance identification with the presenter that is credible and seemingly not staged. Topic areas will be congruent with the three other proposed interventions.

Study Design

The basic study design for this protocol involves administering a pretest to all who have agreed to participate. Individuals will then be randomized to either a treatment group or to the control group. Participants in the treatment conditions will then complete the intervention. At 6 months, individuals will receive the post test assessment. At that time individuals who were in one of the three treatment groups will receive a "booster." The researchers have chosen to use the HRAPIC counseling as the booster because they believe it will be the most effective of the interventions, for reasons explained previously. Those who were randomized into the control group will not receive a booster. At 12 months, all groups will receive a follow-up assessment.

Pretest and follow-up assessment measures will include a behavioral assessment, based on the SBS, psychosocial measures (a subset of those used in the HIV Biopsychosocial Study), and a brief cognitive assessment (also a subset of measures used in the HIV Biopsychosocial Study).

Significant Findings : Not applicable

Patient Visits: 45 patient focus groups
5 staff or health care provider groups

Sites: HIV Neurobehavioral Research Center, San Diego, CA
National Naval Medical Center, Bethesda, MD
Walter Reed Army Medical Center, Washington, DC

**RV89-"Obstetricians-Gynecologists' (OB-GYN) Affective Reactions to
Uncertainty and Discussing HIV/AIDS during Routine Gynecologic Care"-**

Protocol Description:

The purpose of this study is to measure the influence of obstetricians-gynecologists' (OB-GYNs) anxiety due to clinical uncertainty, and patient, physician, and organizational characteristics in explaining differences in the physicians' selection of human immunodeficiency virus (HIV) related educational activities during routine gynecologic care. The study will examine the impact of patient factors, (e.g., ethnicity, sexual history and practices), physician factors (e.g., gender, age, job position, staff, fellow or resident), and organizational factors (e.g., institutional policies and colleague-dependent norms) on physicians' practices of HIV/AIDS prevention activities.

Specific objectives of the study are :

- a. Determine the impact of anxiety due to uncertainty, and patient, physician, and organizational factors on physician's use of HIV-related patient education.
- b. Determine the interactive effects of OB-GYNs, i.e., anxiety due to uncertainty and patient, physician, and organizational factors.

Technical Approach:

Questionnaire Development and Pilot Study

The final survey is a 5-section, self-administered questionnaire. The questionnaire covers factors found in the literature to be most associated with OB/GYN's participation in STD/HIV prevention (e.g.; demographics; comfort in discussing STD/HIV prevention during routine care; their HIV prevention attitudes, training and practice; their anxiety due to clinical uncertainty; and the

likelihood that they would perform specific HIV-related educational activities in response to two hypothetical patient care encounters).

A pilot study was conducted in November 1993 with Army internal medicine physicians to test the 5 sections of the instrument for internal consistency and validity. Forty-five out of 200 internists (22.5%) completed the questionnaire; no attempt was made to encourage participation. Participants were encouraged to suggest changes to the questionnaire. Based on pilot data and participant suggestions, several changes were made to the design to improve reliability, clarity and focus.

Significant Findings:

Analysis of pilot data revealed that fewer than 20% of respondents reported educating their patients routinely about STDs or HIV, even though the majority of respondents indicated that they were only "fairly sure" that their patients were not at risk for HIV infection. There were few differences among respondents in the number of HIV-related educational activities selected in response to the hypothetical "high" risk patient. However, significant differences were found in the number of HIV-related educational activities selected by respondents in response to the "low" risk patient. The type of respondent most likely to perform HIV-related educational activities with a "low" risk patient was older, more knowledgeable, and trained on-the-job. This physician also was more comfortable dealing with sensitive sexual topics. Finally, this respondent perceived much more organizational support, and fewer constraints, than respondents who selected fewer HIV-related educational activities in response to a hypothetical "low" risk patient. Data collection in the main study has begun and is expected to be completed by the end of this year.

Number of Participants Enrolled: 45 in pilot study

275 questionnaires were mailed to Army OB-GYN staff, fellows, and residents, the total population of Army physicians listed in PERSCOM records under specialty code J60 ("obstetrician-gynecologist"), and practicing and training in the obstetrics-gynecology departments in medical settings CONUS and OCONUS. Two follow-up mailings are planned to encourage maximum participation.

Patient Visits : Not applicable

Sites: All Army medical facilities, CONUS and OCONUS.

RV96-"Assessment and Modification of Sexually Transmitted Disease (STD) Risk-Relevant Behaviors at the United States Naval Station, San Diego"-

Protocol Description:

In this study, interventions are designed and evaluated to decrease HIV risk-relevant behavior among a group of US Navy personnel in San Diego, at high risk for exposure to HIV. The objectives of this study are:

- a. Assess the risk factors in the San Diego Navy population using surveys and focus groups.
- b. Develop two interventions based on the results of the surveys and focus groups.
- c. Evaluate the effectiveness of the two focus groups and the standard intervention presently offered at the STD clinic.

Technical Approach:

The survey will be based on the Army Wide AIDS/HIV Survey, with additional questions to assess risk factors that are unique to the Navy population in San Diego. These additional questions will be based upon the focus group discussions.

The survey will be administered using a dual frame, systematic random sampling technique. Subjects will be recruited in (1) the physical examination area and (2) the STD clinic of the Branch Medical Clinic in San Diego. The subjects will be divided into two groups: those who have had a STD diagnosis within the past year and those who have had no STD diagnosis within the past year. About 900 subjects will be recruited over a five month period.

Analyses of the results of the surveys and the focus groups will reveal which specific risk factors are prevalent among sailors who have had a STD recently, and which risk factors are prevalent throughout the more general San Diego Navy population. Two interventions will be designed to address the needs identified in the surveys and focus groups. The two interventions and the standard counseling and education presently offered will be compared to determine their relative effectiveness in reducing STD/HIV risk factors.

Significant Findings:

This protocol is in the approval process. It received Retrovirus Clinical Review Committee approval on 08 June 94. The protocol for the initial phase of the study was submitted for Tri-Service Scientific Review on 21 July 94. A response from the scientific review committee was received in mid-August. A

memorandum replying to the scientific review has been submitted to the committee.

Number of Patients Enrolled: Not applicable

Patient Visits : Not applicable

Site: This is a single site study at the Branch Medical Clinic, US Naval Station, San Diego, California. The HIV Neurobehavioral Research Center, San Diego, California and the Naval Health Research Center, San Diego serve as collaborators.

BEHAVIORAL PREVENTION PROGRAM AREA COLLABORATIVE EFFORTS

The following set of studies reflect the current efforts of the Behavioral Prevention Program to support other HIV Research Program Areas and military operational Efforts:

Addendum to RV43-"Prospective Study of the Emergence of Zidovudine Resistance in Patients Infected with Human Immunodeficiency Virus who are Treated with Zidovudine (ZDV): Behavioral Addendum"-

Protocol Description:

This addendum is conducted in collaboration with the HIV Disease Prevention Program Area. The purpose of this behavioral addendum is to provide information about attitudes, beliefs and expectations concerning the use of ZDV and the impact of learning one was becoming resistant to the effects of ZDV. Specific objectives were as follows:

- a. Demonstrate the predictive validity of a self report measure of ZDV adherence.
- b. Determine patients' attitudes, beliefs, and expectations about ZDV treatment, including personal beliefs concerning HIV treatment efficacy, when to change treatment agents, one's ability to transmit HIV while on treatment.
- c. Determine the impact of these beliefs, expectations and attitudes, and affective state on behavioral intentions concerning adherence with treatment recommendations.

- d. Determine the impact on adherence of release of clinical data to patients.

All participants in the ZDV resistance study (RV43) have been invited to participate in the study. Upon consent, individuals complete a brief questionnaire concerning attitudes, expectations and perceptions of their treatment with ZDV. The questionnaire has been modified from the Perception of Treatment Questionnaire used in the Tri-Service Biopsychosocial Study. Criterion for adherence will be based on an algorithm comprised of biomedical variables. An additional brief form of the Perception of Mood States (POMS) also is administered.

Significant Findings: Data analysis has not yet begun

Number of Patients Enrolled: 33

Patient Visits: 33

Sites: National Naval Medical Center, Bethesda, MD
Walter Reed Army Medical Center, Washington, DC

Addendum to RV79-"A Double Blinded, Randomized Trial Comparing Zidovudine (ZDV) vs. ZDV+ Didanosine (ddI) vs ZDV+ ddI+ Nevirapine in Asymptomatic Patients on ZDV Monotherapy Who Develop a Mutation at Codon 215 of HIV Reverse Transcriptase in Serum/Plasma Viral RNA: Behavioral Addendum"-

Protocol Description:

This addendum is conducted in collaboration with the HIV Disease Prevention Program Area. The Behavioral Addendum to RV79 will assess through the use of questionnaires, participants' attitudes, beliefs, and expectations about participating in this clinical trial, including issues about being on placebo and changing treatment agents. The specific objectives are as follows:

- a. Demonstrate predictive validity of a self report measure of ZDV adherence.
- b. Determine patients' attitudes, beliefs, and expectations about ZDV treatment, including personal beliefs concerning HIV treatment

efficacy when to change treatment agents, ability to transmit HIV while on treatment, and assumptions about taking a placebo.

- c. Determine the impact of these beliefs, expectations, attitudes, and affective state on behavioral intentions concerning adherence with treatment recommendations.
- d. Determine the impact of patients learning they may be ZDV resistant on their attitudes to treatment and decisions about changing treatments.

Participants at the Metropolitan D.C. Area site who are participating in the parent protocol (RV79) are asked to complete questionnaire at 6 month intervals for approximately a 2 year period. Questionnaire administration is planned to coincide with clinical visits where laboratory data and data on pill counts are gathered. The questionnaire is a modification of other Behavioral Prevention Program Area questionnaires concerning perceptions of treatment. Data from the questionnaire will be analyzed in concert with relevant laboratory and pharmacy data in order to meet the objectives of this study. This study is being considered by the local sites' human use committees; data collection will commence immediately upon approval.

Significant Findings: Not applicable

Number of Patients Enrolled: Not applicable

Patient Visits : Not applicable

Sites: National Naval Medical Center, Bethesda, MD
Walter Reed Army Medical Center, Washington, DC

RV97-"The Use of Patient Functional Status Measurement to Predict Patient Acceptance/Rejection of Antiretroviral Drug Regimen"-

Description of Protocol and Objectives:

This protocol is being conducted in collaboration with the HIV Disease Prevention Program Area. The purpose of this study is to understand how changes in functional status following initiation of antiretroviral treatment will affect continued patient acceptance of and adherence to medication regimens. The objectives of this study are:

- a. Determine whether functional status measures can predict willingness to continue use of a prescribed antiretroviral medication by HIV-infected patients at one, three, six and 12 months after initiation of treatment.
- b. Determine whether functional status measures can predict adherence to prescribed antiretroviral medication treatment and to the protocol by HIV-infected patients at the same time intervals after treatment initiation.
- c. Determine the relation between expressed acceptance of and actual adherence to prescribed treatment.
- d. Determine the impact of acute medication toxicities on functional status.
- e. Determine the impact of HIV-associated complications and opportunistic infections on functional status.

Technical Approach:

150 HIV-infected individuals will be studied. One hundred will be initiating antiretroviral treatment, and 50 will be changing or adding to their current antiretroviral treatment. Data from this study will contribute to understanding factors that affect early acceptance of and adherence to prescribed treatment. By improving medication acceptance and adherence, HIV-infected military personnel will, potentially, be able to maximize the length of time that they remain asymptomatic and on active duty. Self-report questionnaire measures of functional status (MOS 30-item HIV Questionnaire [Wu et al, 1991]; Functional Assessment of HIV Infection [Cella, 1994]), affective status (Beck Depression Inventory [Beck & Steer, 1987]; State portion of the State-Trait Anxiety Inventory [Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983]), cognitive status (Abbreviated Awareness of Functioning Questionnaire [Mapou, 1993, August]), and Perceptions of Treatment [Nannis, Brandt, Temoshok, Kendall, & Jenkins, 1993, June]) will be administered. Adverse events during treatment will be recorded. Acceptance of treatment will be measured by questionnaire and by noting if a subject returned for a scheduled medication refill. Adherence to treatment will be measured by questionnaire, pill counts, and changes in mean corpuscular volume (zidovudine only). Subjects will be evaluated at baseline and after one, three, six, and twelve months of treatment. Analyses will be used to predict acceptance and adherence at each follow-up point based on functional status and adverse event reports at the previous assessment point(s). Currently, revisions are being made in the protocol as recommended by the Scientific Review Committee.

Significant Findings: Not applicable

Number of Patients Enrolled: Not applicable

Patient Visits: Not applicable

Sites: Walter Reed Army Medical Center, Washington, DC
National Naval Medical Center, Bethesda, MD

THAILAND COLLABORATION

"Behavioral Side Effects and Behavioral Factors in Study Recruitment, Participation, and Adherence"-

Although not part of a formal protocol, the Behavioral Prevention Program Area has been providing behavioral scientific consultation to the Preventive Vaccines Program Area and the planned prophylactic vaccine trials. The main purposes of the consultation are to: 1) develop and evaluate methods for the recruitment, accrual, and retention of protocol participants; and 2) develop and evaluate behavioral intervention programs to support protocol participation and adherence, as well as to prevent behavioral side effects. These are achieved through consultations in HIV Research Program protocols and in the development of local Thai contracts that are assisting with the vaccine development effort. In addition, the Behavioral Prevention Program Area staff serves as the behavioral science liaison between the Preventive Vaccines Program Area and the Royal Thai Army.

IV. CONCLUDING REMARKS

The Behavioral Prevention Program Area has a highly challenging mission. The HIV education and prevention efforts of the three military services have a marked and immediate need for behavior change interventions. This need involves multiple at-risk populations and groups throughout the military's training and deployment cycles, with special needs in certain areas or situations. For this reason, multiple interventions must be designed, developed, and evaluated in field settings. In addition, military priorities often place time constraints on both the initial evaluation of these interventions, and if successful, their subsequent operational utilization. Behavioral Prevention Program Area interventions often must be administered in under 1 hour with an expectation that behavioral changes will be maintained long term. In addition, interventions must be sensitive to the rules and regulations inherent in the military setting. The researchers have been highly ambitious and creative in developing not only one, but several, interventions which meet these unique requirements. Having completed this task, the Behavioral Prevention Program Area now is poised to assess whether these efforts have been successful.

The approach taken by Behavioral Prevention Program Area has placed this program area at the forefront of HIV behavioral intervention development. The two best examples of this are the database approach to interventions and an interdisciplinary perspective. This Program Area's use of a database approach to intervention preceded that of the civilian world. Unlike the NIH multisite study which fielded interventions without the benefit of prior descriptive information and found the initial interventions to be minimally effective, the Behavioral Prevention Program Area bypassed this by initiating a data-driven approach to intervention development. Further, the focus on seropositive individuals as a key part of the prevention agenda and the multidisciplinary approach to understanding behavior (the integration of psychosocial, behavioral, neuropsychological and biological factors) was recently reaffirmed as the direction that HIV prevention research should be taking by the recent report of the Institutes of Medicine.

Work from this Program Area has been presented and well received at major national and international conferences. As a direct measure of productivity, this Program Area has authored or co-authored 45 presentations and publications. At present, 5 innovative interventions are ready for evaluation and two more are in the early stages of development. The latter are computer driven intervention applications, the format of which have been pretested with and received positively by HIV-infected individuals. These interventions are innovative not only in their technologies but in the messages they emphasize. The scientists in this program area envision the next year to be a highly productive year, fielding and testing our new intervention technologies.

Additionally, the Behavioral Prevention Program Area distinguishes itself by its collaborations with drug and vaccine trial efforts, as recommended in this year's AIDS/HIV International Conference in Japan. Measures have been developed to better understand the impact of psychological factors in individuals' willingness to participate

in trials and in their adherence to trial guidelines. This program area continues its commitment to support other HIV Research Program Areas in their priority work.

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HIV DISEASE PREVENTION

USAMRMC Program Area Coordinator:

HJF Scientific Director:

CAPT Douglas Mayers, MD, MC, US Navy

Daniel St. Louis, Ph.D.

Program Area Summary:

This program area concentrates on exploring promising antiretroviral drugs for clinical efficacy trials in patients with early stage HIV disease. A recent new focus in this area is the development of efficient gene delivery systems to be ultimately used in treatment of early-stage HIV-1 disease.

Human Use protocols

Protocol Number	Title (Abbreviated)	Principal Investigator
RV28	Pharmacology Database	Cortese
RV43	Prospective Study of AZT Resistance	Mayers
RV77/78	Lymphoid Tissue Studies	Anderson/Turnicky
RV79	Codon 215 - ACTG 244	Mayers
RV88(P)	DMP 412 HIV Protease Inhibitor	Wagner/Mayers
RV94(P)	Surrogate Marker Trial	Bernstein
RV97(P)	Functional Status Measurements	Mapou
RV100(P)	Adoptive Cellular Transfer	June/Mayers/St. Louis
RVOI8	Prophylaxis of MAC	Berman
CRV7	Prospective Lymphocyte Collection and Banking	Harris

Laboratory Work Units/Projects

Project Title (Abbreviated)	Principal Investigator
Chemotherapy/Chemoprophylaxis:	
Drug Resistance/Virology Studies	Bernstein/Mayers
Viral Burden Measurements	Vahey

Immune Function	Hickey/June
Pharmacology	Hendrix/Cortese
Functional Status Measurement	Mapou
Cellular Pathology:	
Cellular Pathology/Tissue Studies	Anderson
Detection of HIV-1 Cells Using PCR and Fluorescent <i>In Situ</i> Hybridization	Wegner
Detection of HIV-1-Infected Cells by Flow Cytometry	Perfetto
Viral Burden - RNA and DNA PCR Analysis in Purified Sorted Cell Populations"-	Anderson
Gene Therapy:	
Molecular and Genetic Characterization of MoMuLV and HIV-1	St. Louis
Prophylactic and Immunotherapeutic Vaccine Strategies	St. Louis
Assessing Anti-HIV Antiviral Gene Therapy Strategies	Mosca/St. Louis
Assessing Anti-HIV Antiviral Strategies in Hematopoietic Stem Cells	Mosca
Culture Systems	June

I. OVERVIEW

The HIV Disease Prevention Program Area has three primary components. The first component, the HIV Chemotherapy/Chemoprophylaxis group works in collaboration with other government agencies and private industry to test promising antiretroviral drugs for clinical efficacy trials in military HIV-infected populations. In conjunction with the AIDS Clinical Trial Group (ACTG), this group has developed an HIV drug susceptibility assay to examine both the potency of promising new antiviral drugs and to monitor the susceptibility of virus derived from patients on therapy to drug. The group continues to develop assays to assess responses to interventions in patients with early stage HIV disease in collaboration with Dr. Carl June's laboratory at NMRI, Dr. Maryanne Vahey in the Intervention Assessment Program Area and Dr. Owen Weislow at SRA Technologies. Assays under development include: syncytium-inducing (SI) phenotype, genotypic assays for drug resistance, a screening assay for phenotypic AZT resistance, quantitative HIV RNA PCR, and measurements of cellular immune function including T cell signaling, CD3 stimulated expression of CD69, and apoptosis measurements. The Behavioral Prevention Program Area is integrating functional status measurements into ongoing clinical trials. A Pharmacokinetic/Pharmacodynamic workunit to determine optimal dosing schedules for clinical trials has been established.

The second component, the Cytopathology/Flow Cytometry group, is working to determine and validate tissue-based surrogate markers for disease progression and to determine if these markers can be used to monitor efficacy of drug or gene therapies administered to patients.

The third component, the Gene Therapy group, is working to develop efficient gene delivery systems capable of transducing and expressing genes encoding HIV and SIV antigens to immunize uninfected individuals or augment immune responses in infected individuals and anti-HIV/SIV antivirals to treat early-stage HIV-infected personnel.

II. RESEARCH GOALS AND OBJECTIVES

In support of the HIV Research Program's primary mission of reduction of HIV incidence among military medical beneficiaries, the HIV Disease Prevention Program Area has the following goals and objectives:

- A. Conduct clinical trials of chemotherapy and gene therapy agents focused on the prevention of progression of HIV disease in patients with early-stage disease.
- B. Develop chemotherapy and gene therapy strategies to prevent HIV infection following exposure to HIV.
- C. Develop systems to monitor the susceptibility/resistance of HIV isolates to the available chemotherapy and gene therapy agents in the military and military-associated populations.
- D. Develop systems to monitor the efficacy of interventional therapies in trials of patients with early-stage HIV disease.
- E. Develop regimens to prevent the emergence of drug-resistant HIV strains or to treat patients with drug-resistant strains when they emerge.

III. TECHNICAL APPROACH

The following section describes the clinical studies, human use protocols and laboratory projects which comprise the technical work in this Program Area:

Clinical Studies

RV43- "Prospective Study of the Emergence of Zidovudine (AZT) Resistance in Patients Infected with the Human Immunodeficiency Virus (HIV) who are Treated with AZT"-

Protocol Description:

A cohort of 100 patients with CD4 counts <400 cells/mm³ on zidovudine monotherapy was evaluated every three months for a period of 3 years. Patients had a screening history and physical exam along with T cell subsets, p24 antigen and drug level determinations. HIV isolates at each time point were evaluated for susceptibility to AZT, ddI and ddC, along with syncytial phenotype. Multiple aliquots of plasma and peripheral blood mononuclear cells (PBMCs) were stored from each time point for further studies. The objectives of the study were:

- a. Determine the time course, frequency and clinical parameters associated with the development of AZT resistance in HIV isolated from patients on AZT.
- b. Determine if there exists a level of AZT resistance, measured *in vitro*, which correlates with clinical deterioration in patients who are receiving AZT.
- c. Develop a repository of frozen HIV infected PBMC with resistant virus for future studies into the molecular basis of dideoxynucleotide resistance.

Significant Findings to date:

Several factors predicted the early emergence of AZT resistant virus including:

1. Low CD4 cell counts at initiation of AZT therapy
2. Presence of a syncytium inducing (SI) HIV isolate
3. Plasma HIV RNA > 105 copies/ml

Factors which predicted development of opportunistic infection or death included:

1. Low CD4 cell count at study entry

2. Plasma HIV RNA > 105.5 copies/ml
3. AZT IC50 > A.0 μ M at study entry

In addition, this protocol demonstrated that:

- CD4 cell counts remain stable in patients with AZT-susceptible virus (by phenotype or codon 215 determination) for periods as long as 4 years.

- CD4 cells decline by 120 cells/mm³ in the year preceding the emergence of *in vitro* AZT resistance (defined as IC50 > 1 μ M AZT).

- Mutations in reverse transcriptase codon 215 could be detected by polymerase chain reaction in patient plasma or PBMC at a time when the patient's CD4 count remained near baseline from the start of therapy.

- After the emergence of a population of HIV with mutant codon 215, 50% of patients will drop their CD4 count by > 50% from baseline, and 25% will have CD4 cell counts < 50 cells/mm³.

- For patients who switched from AZT to ddI, no clinical, immune, or virologic parameters predicted the subsequent change in plasma HIV RNA. Adverse outcomes were associated with CD4 counts < 100 cells/mm³, baseline plasma HIV RNA > 105.5 copies/ml, and the absence of a decline in plasma HIV RNA after switching from AZT to ddI.

Number of Patients Enrolled: 100

Number of Patient Visits to Date: 654

Sites: Walter Reed Army Medical Center, Washington, DC
National Naval Medical Center, Bethesda, MD

RV77- "Rectal Mucosal and Lymph Node Biopsy of Early-Stage HIV-Infected Patients"-

and

RV78- "Pharyngeal and Lymph Node Biopsy of Early Stage HIV-Infected Patients"-

Protocol Description:

The ability to evaluate therapy in HIV-infected individuals has been hampered by the lack of a suitable animal model or *in vitro* assay capable of reflecting the complex interplay within a human biologic organ system. Additionally, in early disease, HIV staging systems do not accurately reflect prognostic significance of Walter Reed stage 1/2 patients.

These two studies will establish the feasibility of lymph node fine needle aspiration biopsy to measure parameters of virologic and immunopathogenesis in HIV patients with Walter Reed Stage I-II HIV disease. The primary objective of these studies are to evaluate the feasibility of quantitative p24, albumin, CD4, β -globin DNA, HIV DNA and HIV RNA analysis of lymph node cell/fluid specimens obtained by fine needle aspiration of biopsied nodes.

This laboratory utilizes human lymphoid follicular tissue as a model to refine early HIV staging and to monitor therapeutic trials for HIV Research Program investigators. The researchers planned to do this by identifying surrogate markers which are prognostic and capable of assessing intervention therapies. Both surrogate markers and viral burden were quantitated by cell type and tissue compartment of lymphoid tissues.

Significant Findings:

The researchers presented the immunologic profile and viral distribution between two Walter Reed Stage II (WR II) matched nodes and a noninfected, hyperplastic lymph node. A unique feature of this study was the performance of quantitative image analysis (IA) on tissue sections in the evaluation of the immunophenotypic profile. A significant finding was the increase in CD8 positive cells within the germinal centers (GC) of infected nodes. There was a 9-fold increase in the CD8 effector cells over the noninfected hyperplastic node. Follicular areas, which serve as an HIV reservoir, were increased 27% over control tissue when measured by image analysis.

Lymph nodes from HIV positive, WR II patients with a pathologic diagnosis of Persistent Generalized Lymphadenopathy demonstrated differences in the pattern of HIV viral distribution. The variant patterns suggested an altered evolution of disease progression in stage-matched patients. Increases in

individual cells positive for HIV expression were noted in the germinal centers and interfollicular areas of one node. This node also showed involutional changes of the GC. The node lacking involutional features showed a diffuse pattern of HIV expression throughout the GC with less individual cell positivity. This pattern of infection suggests an HIV distribution associated with dendritic reticulum cells.

This study indicates the need for more refined surrogate markers in early stage HIV disease. Monitoring CD8 infiltrates and viral burden within tissue compartments of the lymph node may serve as useful adjuvants to prognosticate disease progression and evaluate treatments of HIV infected patients.

Detailed laboratory findings from the study can be found in the Cellular Pathology Work Unit.

Total Number of Patients to be Enrolled: RV77 -12, RV78 - 6

Site: National Naval Medical Center, Bethesda, MD
Walter Reed Army Medical Center, Washington, DC

RV79/ACTG244- "A Blinded, Randomized Trial comparing Zidovudine (ZDV) vs ZDV + ddI vs ZDV + ddI + Nevirapine in Asymptomatic Patients on ZDV Monotherapy Who Develop a Mutation at Codon 215 of HIV Reverse Transcriptase in Serum/Plasma Viral RNA"-

Protocol Description:

A cohort of 300 asymptomatic patients on ZDV monotherapy with CD4 counts 300-600 cells/mm³ are followed for the emergence of ZDV resistance determined by detection of a mutation of reverse transcriptase at codon 215 in plasma viral RNA. After detection of the 215 mutation, patients are randomized to ZDV, ZDV + ddI, or ZDV + ddI + Nevirapine and followed for subsequent CD4 decline. Primary objectives of the study are:

- a. Validate that alteration of codon 215 of reverse transcriptase in plasma virus precedes the increase in viral burden and decline in CD4 count associated with clinical failure on Zidovudine monotherapy.
- b. Determine whether alternative regimens of antiretroviral agents alter the course of viral burden increase and CD4 decline associated with clinical failure on Zidovudine monotherapy when treatment is changed on the basis of plasma RNA PCR results.

Significant Findings:

This is the first joint chemotherapy trial between the ACTG and the HIV Research Program. This study offers the possibility of rationalizing ZDV therapy by the use of plasma RNA PCR to detect emergence of ZDV resistant virus prior to modification of antiretroviral therapy. The study will prospectively relate emergence of ZDV resistance, viral burden and syncytial phenotype with CD4 decline to address the relationship of viral parameters to clinical decline. This study was designed based on the results from RV43 and opened for enrollment in February 1994. There are 33 DoD patients and 79 ACTG patients enrolled. As the study has progressed, the HIV Research Program has taken on a larger role in processing and testing the specimens for the 215 mutation.

Total Number of Patients to be Enrolled: 100 DoD and 200 ACTG

Total Enrolled in Protocol: 112 (33 DoD patients, 79 ACTG patients)

Additional (future) Number of Patient Visits Projected: 2700 (DoD)

Sites: National Naval Medical Center, Bethesda, MD
Walter Reed Army Medical Center, Washington, DC
Wilford Hall Medical Center, San Antonio, TX
Brooke Army Medical Center, San Antonio, TX
Fitzsimons Army Medical Center, Aurora, CO

RV88- "A Safety, Tolerance, Pharmacokinetic and Pilot Efficacy Study of Multiple Doses of DMP 412 in HIV-Infected Patients with AZT Active Control"-

Protocol Description:

This is a double-blind, randomized, comparative agent controlled, multiple escalating dose Phase I/II trial of DMP 412 (an oral HIV protease inhibitor) versus AZT in 48 patients with CD4 counts ≤ 500 cells/mm³ and p24 AG ≥ 35 pg/ml. Patients will either receive DMP 412 250 mg TID, 500 mg TID, 750 mg TID, or AZT 200 mg three times a day. One half of the patients on each arm will have a history of virus evaluated as resistant to AZT. Patients will be observed for up to 8 weeks and offered a 24 week open label treatment extension with DMP 41b. Specific objectives include:

- a. Determine the pharmacokinetic, tolerability and safety profile of DMP 412 upon administration of multiple ascending doses to HIV infected patients and in combination with AZT.

- b. Determine doses of DMP 412 which produce measurable antiviral activity.
- c. Prospectively survey for the emergence of *in vitro* resistance to DMP 41b.

Significant Findings:

This study is on hold due to formulation problems with the original drug DMP 32c. The company has switched to a second generation compound DMP 412 for use in a proposed trial in early 1995.

Total Number of Patients to be Enrolled: 48

Sites: National Naval Medical Center, Bethesda, MD
Balboa Naval Hospital, San Diego, CA
Walter Reed Army Medical Center, Washington, DC
Wilford Hall Medical Center, San Antonio, TX

RV94- "A Pilot Study of the Ability of a Panel of Surrogate Markers to Measure the Antiviral Activity of Zidovudine in Early Stage HIV Infected Volunteers"-

Protocol Description:

The testing of new antiretroviral agents in humans would be simplified if a given marker or panel of markers was available that was shown to be valid in assessing the efficacy of antiretroviral intervention in patients with early-stage HIV disease. This protocol was designed to address this issue; specific objectives include:

- a. Measure the impact of zidovudine monotherapy versus placebo on various peripheral blood surrogate markers in patients with early stage HIV infection.
- b. Measure the effect of zidovudine monotherapy on tissue viral burden as assessed by lymph node biopsy and fine needle aspirate.
- c. Establish a repository bank of samples to be used in the future to validate new measures of antiretroviral activity in patients with early stage HIV disease.

Significant Findings: This study is currently in Scientific Review.

Total Number of Patients to be Enrolled: 48

Sites: National Naval Medical Center, Bethesda, MD
Walter Reed Army Medical Center, Washington, DC

RV97-"The Use of Patient Functional Status Measurement to Predict Patient Acceptance-Rejection of Antiretroviral Drug Regimens"-

This protocol is a collaborative effort between this program area and the Behavioral Prevention Program Area. Detailed information on this protocol can be found in that Program Area's section.

RV100- "A Phase I Dose-Escalation Study of Polyclonal CD4 T Cell *Ex Vivo* Expansion for Immune System Restoration of HIV Infection"-

Background:

To the extent that opportunistic infection and neoplastic complications of HIV-1 infection are due to loss of CD4 cells, the restoration of CD4 cell function would be expected to restore clinical well-being. The immediate applications of this protocol are to establish the technology for a new form of transfusion therapy. This consists of (a) *ex vivo* expansion of CD4 cells from HIV-infected patients, (b) using drug therapy for suppression of viral replication during *ex vivo* cellular expansion, and (c) reinfusing polyclonal CD4 T cells to patients. The potential benefit of this proposed protocol is reconstitution of the T cell repertoire and restoration of T cell function of anergic cells, with clinical benefits of increased CD4 cell number and improved or stabilized immune memory.

The long-term application envisioned is to restore the patient's T cell function with polyclonal T cells that have a survival advantage over noninfected cells. This approach may be life-sustaining, but cannot be expected to be curative, due to the limited lifespan of T cells (months to years) and limited efficacy of current antiviral therapy. Curative approaches can, however, be envisioned if this form of polyclonal CD4 cell therapy is combined with antigen-specific T cells, or with other forms of antiviral therapy.

Protocol Description:

This is a Phase I trial of adoptive cellular transfer to determine the safety of CD4 cell *ex vivo* expansion and reinfusion in HIV-1-infected patients. The endpoints of the study are measures of viral burden and circulating CD4 T cell mass. A second objective of this study is to test escalating doses of CD4 cell infusions, however the maximum tolerated dose of CD4 cells in HIV-infected patients will not be determined in this study.

Long-term Objective: The ultimate goal of this form of therapy is to determine if restoration or normalization of CD4 cell mass in HIV-infected patients improves long-term disease-free survival. Other long-term objectives are to use the information gained from this protocol to provide an experimental basis for future studies involving (a) the infusion of gene-modified CD4 T cells and (b) antigen-specific CD4 T cells.

Significant Findings:

This protocol has been approved by the Retrovirus Clinical Research Committee.

Number of Patients to be Enrolled: 10

Sites: National Naval Medical Center, Bethesda, MD
Naval Medical Research Institute, Bethesda, MD
Walter Reed Army Institute of Research, Washington, DC

CRV-7- "Prospective Collection and Banking of Lymphocytes and Clinical Data on HIV-Infected Individuals taking Antiretroviral Agents"-

Description of Protocol:

This study is an extension of a previous Phase II study of AZT therapy in 220 patients followed by investigators at Fitzsimons Army Medical Center and Denver Health Hospital. Samples of patient sera and cells were collected every 6 months and stored in a central repository. This systematic collection of clinical data and specimens from patients on prolonged antiretroviral therapy (periods exceeding 5 years) has made the repository at Fitzsimons an invaluable resource for drug resistance studies being conducted at WRAIR.

Significant Findings:

- A case control study of patients on AZT monotherapy demonstrated the association of AZT resistance with clinical decline. A manuscript of this study is in preparation.
- A natural history study of the entire cohort of patients showed that initiation of AZT therapy delays Walter Reed stage progression by approximately 500 to 800 days, but subsequent stage progression occurs at similar rates in untreated patients and patients progressing on therapy.
- A set of samples obtained from recent HIV seroconverters in the cohort is being validated for use in a retrospective study of the incidence of transmission of AZT resistant virus.

Number of Patients Enrolled: 532

Site: Fitzsimons Army Medical Center (FAMC), Aurora, CO

RVOI8-"A Double-Blind, Placebo Controlled, Parallel Group, Multi-center Study for the Prophylaxis of Mycobacterium Avium Complex with Azithromycin in HIV Infected Patients"-

Description of Protocol:

This protocol was a twenty-four month prophylaxis study using a weekly dose of Azithromycin as possible protection against Mycobacterium Avium Complex (MAC). HIV-infected patients with CD4 counts <100 were randomly assigned to placebo or azithromycin (1200 mg weekly) groups. Patients were evaluated monthly for the major endpoints: mycobacteremia, other bacterial infections, signs/symptoms of drug toxicity.

Over 70 patients at eight sites were enrolled and some patients have already reached endpoints in this double blind study.

Significant Findings:

Although this protocol continues to be a low priority protocol for the HIV Research Program, clinicians are in agreement to continue the current patients on protocol.

Sites: Eisenhower Army Medical Center, Ft. Gordon, Georgia
Fitzsimons Army Medical Center, Aurora, Colorado
Walter Reed Army Medical Center, Washington, DC

Wilford Hall Medical Center, San Antonio, Texas
National Naval Medical Center, Bethesda, Maryland
Brooke Army Medical Center, Ft. Sam Houston, Texas
Naval Hospital at San Diego, California
Womack Army Medical Center, Ft. Bragg, North Carolina

Chemotherapy/Chemoprophylaxis Work Unit

The HIV Chemotherapy/Chemoprophylaxis group, in collaboration with other government agencies and private industry, tests promising antiretroviral drugs for clinical efficacy trials in the military HIV infected population. In conjunction with the AIDS Clinical Trials Group (ACTG), the HIV Chemotherapy/Chemoprophylaxis group has developed an HIV drug susceptibility assay to examine both the potency of promising new antiviral drugs and to monitor the susceptibility of patient isolates to these new drugs. To improve understanding of the development of drug resistance, researchers in this program area have begun a sequential study of different viral compartments (plasma, monocytes, and CD4 lymphocytes) in patients on RV43. In collaboration with Dr. Carl June, Dr. Maryanne Vahey (Intervention Assessment Program Area), Dr. Owen Weislow (SRA Technologies), and Dr. Robert Mapou (Behavioral Prevention Program Area), the group is actively pursuing the development of assays to assess responses to interventions in early stage patients. Such assays include a screen for syncytium inducing (SI) phenotype and genotypic and phenotypic analysis of drug resistance. Methods to measure viral burden in these patients include quantitative HIV RNA PCR. Measures of cellular immune function under development include T cell signaling, CD3 stimulated expression of CD69, and apoptosis. In keeping with the growing importance of quality of life issues, the group will integrate functional status measurements into all ongoing clinical trials. A Pharmacokinetic/Pharmacodynamic workunit to determine optimal dosing schedules for clinical trials has also been established.

Extensive research is being done to fully understand the molecular basis of drug resistance. Methods are being developed with private industry (Affimetrix) to perfect rapid sequencing. This will permit rapid analysis of serial patient isolates for ongoing clinical trials. Knowledge from the development of this technology will be applied to determine the clinical significance of specific mutations. Subsequently, site-directed mutagenesis can be used to generate specific drug-resistant mutants which can be inserted into expression vectors. This in turn will enhance development of methods to prevent or reverse clinically significant resistance.

Technical Approach: Drug Resistance/Virology

"In vitro Assay Development"

Protocol Description:

The Chemotherapy program has an extensive involvement in collaboration with SRA Technologies, NIAID, and industry (via CRADAs) to develop and validate *in vitro* assays in support of interventional trials in patients with early-stage HIV disease. Assays have been developed to assess phenotypic and genotypic drug resistance and syncytium-inducing virus phenotype. Current efforts involve rapid screening assays for drug resistance and methods to screen stretches of the HIV genome for resistance mutations. Specific objectives include:

- a. Develop assays for viral phenotypic and genotypic properties, especially drug resistance, for use in interventional trials and epidemiologic studies.
- b. Validate the utility of viral phenotypic/genotypic assays in the management of patients with early-stage HIV disease.

Significant Findings:

The following technologies were developed:

- Microtiter-based drug susceptibility assay
 - Tube-based rapid screening assay for drug resistance
 - Microtiter-based SI/NSI assay
 - PCR detection of RT codons 215/74 (AZT/ddI resistance)
 - Sequencing of drug-resistant HIV RT gene
-

"The Timing and Development of Codon 215 Mutations in Plasma, CD4, and CD14 Cells from HIV-Infected Patients on Zidovudine"-

Protocol Description:

Since monocytes and T cells metabolize zidovudine differently, the researchers wanted to determine whether either of these cell types select for the 215 mutation in patients treated with zidovudine. Specific objectives include:

- a. Compare the development of the reverse transcriptase codon 215 mutation in HIV-1 derived from plasma, CD4 cells and monocytes (CD14 cells) of patients treated with zidovudine.
- b. Determine whether different cell types select for different variants of HIV-1 on the basis of specific regions in the reverse transcriptase gene and or the V3 loop, using patient isolates.

Significant Findings:

- Of the five patients studied thus far, two have resistant virus and three have sensitive virus in plasma, CD4 cells and CD14 cells.
- One patient has sensitive virus in both cell populations and has a mixed virus population in the plasma.
- The codon 215 mutation is concordant in CD4 and CD14 cells in these patients. These and additional patients will be followed sequentially to determine the timing and frequency of the mutation in plasma, CD4 cells and monocytes.
- A reverse transcriptase gene has been sequenced from prototypic monocyctotropic virus for comparison with the patient isolates.

Technical Approach: Viral Burden Measurements

Viral Burden Measurements - HIV RNA

Project Description:

This is a collaborative project with the Intervention Assessment Program Area. Specific objectives include:

- a. Determine the relationship of baseline levels of plasma HIV RNA to development of HIV drug resistance and adverse outcomes in

patients on antiretroviral interventions both as a single variable and in combination with a panel of surrogate markers.

- b. Determine the relationship of changes in levels of plasma HIV RNA to the development of HIV drug resistance and adverse outcomes in patients on antiretroviral interventions both as a single variable and in combination with a panel of surrogate markers.
- c. Determine the utility of measurement of plasma HIV RNA, as a component of an integrated panel of surrogate markers, in determining the efficacy of interventions in patients with early stage HIV disease.
- d. Determine whether measurement of plasma HIV RNA has utility in the management of HIV-infected patients on antiretroviral therapy.

Significant Findings:

- Plasma HIV RNA is strongly associated with development of drug resistance, opportunistic infections and death in patients on long term antiretroviral therapy.
- Failure to decrease the plasma HIV RNA when switching from AZT to ddI is associated with adverse events on ddI therapy.

Technical Approach: Immune Function

"Use of Signal Transduction in HIV-Infected Patients to Monitor Immune Status and Response to Therapy"-

Protocol Description:

Previous work from this laboratory demonstrated a specific defect in cellular signaling induced *in vitro* in human T lymphocytes that survive after infection with HIV-1. The purpose of these studies was to determine whether calcium or other markers of cellular signal transduction might be useful as surrogate markers in the management of military patients with early stage infection. Objectives include:

- a. Determine whether a panel of cell surface markers plus signal transduction and apoptosis assays can be used to assess immune function in patients (pts) with early-stage HIV-1 disease. Eight

patients have been entered under RV2 (see page 192 for further discussion on RV2) for the first pilot study as of August 18, 1994.

- b. Determine which surrogate markers may be applicable to cryopreserved cells. If cryopreserved cells can be used, then a natural history study can be performed from the various banks of cells that are available.
- c. Prospectively determine the relationship of surrogate markers to viral burden. In addition, examine immune parameters in subsets of patients, such as rapid progressors and nonprogressors.
- d. Assess whether these immune parameters can be used as prognostic markers in chemotherapy and gene therapy protocols.

Significant Findings:

Asymptomatic patients with CD4s ranging from 400 to 732 cells/mm³ and normal Delayed Type Hypersensitivity skin testing results were evaluated (n=15). Immune function was monitored through the expression of CD69 induced by anti-CD2/CD28 Mab in whole blood 4 hour assays, and through flow cytometric measurements of intracellular calcium mobilization to CD3 mediated signals. Spontaneous and anti-T cell receptor (WT31) induced apoptosis was evaluated by terminal deoxy-nucleotidyl transferase (Tdt) biotin assays. T cell subsets identified as CD3+ CD28+ CD4+ and CD3+ CD4+ B7+ were also assessed. Other findings included:

- Suppressed calcium mobilization was detected in 50% of the patients, and ranged from 17 to 88 percent of HIV- controls.
- Suppression was detectable in both CD4 and CD8 cell populations.
- Activation responses of CD69+ CD4+ cells from HIV+ donors were also suppressed ranging only 13 to 30% of normal controls.
- Apoptosis after anti-TCR (WT31) stimulation ranged from 1 to 46% for HIV+ pts, and 1 to 14% for the normal controls.
- Spontaneous apoptosis in HIV+ cell cultures ranged from a.5 to 4b.5% compared to 1 to 15% found in the normal controls.

Technical Approach: Pharmacology

"Correlation of Extra- and Intracellular Zidovudine Pharmacology with Clinical, Virologic, and Immunologic Parameters in HIV-Infected Subjects on Long-Term Zidovudine Therapy"-

Protocol Description:

This project is a sub-study within the completed RV43 dataset. Previous analyses have focused on the relationship of virologic, immunologic, and clinical changes in the study cohort over time. This subset seeks to define the magnitude and possible changes over time of the intracellular, active drug, zidovudine triphosphate, in order to quantify the total active drug exposure in the study cohort subjects. These intracellular drug parameters will then be analyzed for correlations with clinical outcomes, both efficacy and toxicity, plasma drug exposure, and virologic and immunologic parameters previously examined in the study. Plasma drug levels do not correlate with efficacy or toxicity outcomes, due largely to saturable, non-linear activation of the parent drug, zidovudine. Defining these interrelationships should both clarify the active drug concentration-biologic response relationship which will assist in the design of future studies, and provide basic information to guide dose optimization in the clinical setting. Specific objectives include:

- a. Define the intra- and extracellular concentrations of zidovudine and its anabolites and their inter- and intra-individual variability.
- b. Correlate the levels of zidovudine triphosphate with zidovudine plasma levels, time to clinical endpoints, virologic and immunologic parameters.
- c. Model the strength of intracellular zidovudine kinetics as a predictor of clinical, virologic (including anti-viral resistance), and immunologic responses over time.

Significant Findings: This project is in the planning stage.

"In vitro Modeling of Anti-HIV Drug Pharmacokinetics and Pharmacodynamics to Optimize Clinical Dosing"-

Protocol Description:

Simulating the human pharmacokinetics of different dosing regimens for one or more antiviral drugs and observing for anti-HIV activity is the focus of this new project. A modeling system has been developed by collaborators in the Division of Clinical Pharmacology at the Johns Hopkins University School of Medicine whereby a dialysis cartridge is employed to simulate the extravascular and intravascular space, the latter of which holds mononuclear cells in culture. Dose and frequency of candidate antiviral drugs can be simulated in the system and compared for antiviral effect. This technique is a very close approximation to human pharmacokinetics, especially when compared to traditional *in vitro* cell culture drug evaluation using static concentrations of drug. These models could then be used to more rapidly and rationally choose optimal dosing regimens for phase II clinical studies. Specific objectives include:

- a. Define the [intracellular active drug concentration]-[anti-HIV response] relationship for candidate compounds.
- b. Define the optimal dosing frequency based on anti-HIV effect for candidate drugs in the modeling system.
- c. Monitor the system for changes in pharmacokinetics or pharmacodynamics over time.

Significant Findings: This project is in the planning stage.

Technical Approach: Functional Status Measurement

"HIV Disease Prevention Functional Status Measurement Work Unit"-

Protocol Description:

It has been increasingly recognized that patient functional status can be an important indicator of outcome in clinical drug trials. For example, a patient's willingness to accept and remain on medication can be substantially influenced by his or her experience with the medication. The emphasis in this Program Area is on treatment of early HIV-1 disease, when active duty military personnel are asymptomatic and less likely to be willing to tolerate side effects of standard antiretroviral or new treatment regimens than later stage patients may be. Therefore, the researchers are beginning to incorporate functional status

measures (FSM) into outcome assessment of standard clinical treatment and clinical drug trials. In contrast to prior work, however, the researchers are taking a broad, multi-dimensional approach to FSM and are including measures of health-related quality of life, mood state, cognitive functioning, and perceptions of treatment. In addition, objective side-effects will be measured, to provide an external assessment of patient functional status.

Specific objectives include:

- a. Validate FSM against standard biological markers as outcome measures in studies of standard and new treatments
- b. Use FSM to predict problems with medication acceptance, in order to intervene prior to decreased medication adherence

Significant Findings:

- Incorporated Perceptions of Treatment measure into RV43. Data analyses are beginning.
- Incorporated Perceptions of Treatment measure into RV79. Data collection is beginning.
- Began to coordinate FSM efforts with similar efforts in the ACTG and at other HIV research centers.
- Developed standard package of FSM and incorporated into:
 - RV94, to validate FSM against surrogate biological markers of treatment outcome. Study is under final review.
 - RV97, to study the ability of FSM to predict acceptance of standard antiretroviral treatment. Study is under final review.

Technical Approach: Seroconverter Studies

"Public Health/Seroconverter Studies"-

Protocol Description:

The military HIV screening program offers a unique opportunity to monitor the HIV epidemic in real time through studies of newly detected seroconverters. The widespread introduction of dideoxynucleoside agents into

clinical practice with the subsequent development of drug resistance leads to concerns regarding the potential for transmission of drug-resistant HIV isolates. Anecdotal reports have documented transmission of drug-resistant HIV-1 via sexual, percutaneous, and maternal:infant routes. Studies have been established with the military HIV screening programs, the CDC, Johns Hopkins University, NIAID (DATRI 002), and Swiss and Australian investigators to monitor the prevalence and clinical impact of drug-resistant HIV seroconversions. Specific objectives include:

- a. Monitor the prevalence of drug-resistant HIV seroconversions in diverse populations of persons at risk.
- b. Determine the clinical impact of seroconversion with a drug-resistant HIV isolate.

Significant Findings:

- Documented the first AZT-resistant seroconversion with investigators at the University of Minnesota.
- Demonstrated that AZT-resistant HIV-1 seroconversions (215 mutation detected by PCR) increased from 3% in 1988-1991 to 19% in 1993-1994 in the United States and Switzerland.

Collaborative Studies:

1. CPCRA 007/014 - Combination Therapy Resistance Substudy
 2. DATRI 003 - Sequential Lymph Nodes Resistance Substudy
 3. SOCA Trial - Foscarnet-Resistant HIV
 4. Duration of Clinical Benefit of AZT Therapy (Collaboration with Intervention Assessment Program Area)
 5. AZT-Associated Hepatotoxicity/Lactic Acidosis (Collaboration with Intervention Assessment Program Area)
-

Cellular Pathology Work Unit

Overview:

The lack of reliable surrogate markers of HIV disease progression hinders our ability to assess the effectiveness of interventional agents such as chemotherapeutic drugs. The mission of this unit is to design and validate lympho-reticular tissue surrogate markers of viral disease progression and relate their levels to comparable measurements in blood. Two protocols have been designed to fulfill this mission, with an addendum of one of them to further develop fine needle aspiration biopsy.

Cellular Pathology-Technical Approach:

RV77 "Rectal Mucosal and Lymph Node Biopsy of Early-Stage HIV-1-Infected Patients"-

and

RV78 "Pharyngeal and Lymph Node Biopsy of Early-Stage HIV-1-Infected Patients"-

At the time of this report, RV77 finished accrual and was in the final stages of the addenda approval (CID at the NNMC) for a fine needle aspiration biopsy addendum to add 12 patients. RV78 accrued three patients and was currently inactive.

Background:

The ability to evaluate therapy in HIV-1 infected persons has been made difficult by the lack of a suitable animal model and deficiencies in virological and immunological markers capable of reflecting the complex interplay between virus and human. Additionally, in early disease such as WR 1 and 2, HIV staging systems have not shown prognostic power.

Protocol Description:

The Cellular Pathology Laboratory group utilized human lymphoid follicular tissues as a model to refine early HIV staging and to monitor therapeutic trials for HIV Research Program investigators. The researchers plan to do this by measuring levels of viral protein and nucleic acid (RNA and DNA) in tissue biopsies and PBMC from early-stage HIV-infected patients. Measurements will include: p24 antigen levels, quantitation of full-length viral RNA and full-length viral DNA copy number and determination of the number of cells expressing viral RNA. Once feasibility trials have allowed optimization of both biopsy technique and the virological assays for tissue, the viral burden in

the lymph nodes can then be compared to the viral burden in PBMCs derived from blood at timepoints before and after therapeutic drug trials.

Significant Findings:

The relationship between lymphoid tissue viral load and histologic pattern (HP) was investigated with excised peripheral lymph nodes (20 patients), tonsils (1 patient), or spleen (1 patient) from 22 subjects (13 living, 9 autopsies). Eleven patients were early Walter Reed Stage (WRS 2), 2 were mid-stage (WRS 4), and 9 had died. Measurements of frozen section tissue viral load by p24 antigen (p24 Ag) per 10^5 cells (ELISA) as well as expressing cell numbers (ECNs) and diffuse follicular center signal (DFCS expressed as multiples of average expressing cell signal) by HIV RNA *in situ* hybridization per 10^5 cells were matched with follicular histology by light microscopy.

DFCS and p24 Ag were highly correlated ($r=0.687$, $p=0.002$) and highest in follicular hyperplasia with or without lysis, patterns found in all patients with WRS 2 or 4 disease. ECNs were similar regardless of histologic pattern or WRS and did not correlate with p24 Ag ($r=0.03$, $p=0.89$) or DFCS ($r=0.143$, $p=0.53$).

FI - Follicular Involution, FD - Follicular Depletion, FH - Follicular Hyperplasia, FH+L - Follicular Hyperplasia w/Lysis

HP	WRS (n)	p24 Ag/ 10^5 cells	ECN/ 10^5 cells	DFCS/ 10^5 cells
FH	2(8), 4(2)	$3D.3 \pm 3B.0$ SD	$1C.3 \pm 1B.5$ SD	1391 ± 772 SD
FH+L	2(3)	$56.8 \pm 6C.0$ SD	$1B.3 \pm D.0$ SD	1926 ± 655 SD
FI	dead (2)	$B.4 \pm 0.9$ SD	25.8 ± 35.7 SD	1438 ± 585 SD
FD	dead(7)	5.6 ± 8.2 SD	$1D.3 \pm 1C.0$ SD	0

Lymphoid tissue viral load appears highest in early to mid-stage HIV disease and diminishes as lymphoid follicles involute and become depleted. The percentage of lymphoid cells expressing viral RNA was relatively constant across clinical disease stage and histologic pattern.

The potential of lymph node fine needle aspiration (LNFNA) for sampling tissue viral load was evaluated in excised peripheral lymph nodes from 5 patients with early-stage HIV-1 disease (asymptomatic, $CD4$ cells $> 300/mm^3$). While the preponderance ($> 80\%$) of viral RNA detected by *in situ* hybridization was found within follicular germinal centers on lymph node frozen sections (LNFS) and within microfragments found in LNFNA preparations, the number of cells expressing HIV-1 RNA in lymph node sections was similar to the numbers observed in LNFNA. Moreover, the viral RNA and DNA copy number per 10^5 cells determined by quantitative polymerase chain reaction and the levels of p24 antigen per 10^5 also yielded similar values in tissue derived from LNFNA.

and lymph node mononuclear cells from tissue homogenates by Ficoll-hypaque separation (LNMC) and LNFS. Sampling of lymph node viral load by LNFNA appeared to capture viral components associated with both dendritic cells in the follicular germinal centers and the individual cells expressing viral RNA. Because FNA permits the investigator the ability to repeatedly sample the lymph node with minimal patient morbidity, assessment of lymphoid tissue viral load by LNFNA will be validated as an alternative to lymph node biopsy in a clinical setting.

"Determination of Viral Burden in Lymphoid Tissue by *In Situ* PCR"-

Protocol Description:

Current molecular diagnostic techniques for measuring viral burden *in situ* underestimate the actual number of infected cells because of limitations in the sensitivity of these techniques. Also, the emergence of drug-resistant cells cannot be accurately mapped *in situ* in lymphoid cells. Additionally, in the near future, there will be a need to develop techniques to detect gene therapy transduced cells *in situ*.

The CPL is developing technologies for amplification of target DNA *in situ* by the polymerase chain reaction (PCR) followed by fluorescent antibody labeling and flow cytometry detection. Methods are also being developed for *in situ* PCR amplification and detection of target DNA sequences on slides of fixed tissue specimens. The target DNA sequences could be either viral genomic sequences, drug resistance mutation sequences, or gene therapy marker sequences such as the neo gene.

Significant Findings:

The PCR protocols being developed by the CPL have achieved cell recovery percentages more than 3 times greater than previously published work. Strategies pursued so far include PCR followed by hybridization with digoxigenin-labeled probes, followed by detection with fluorescein-conjugated anti-digoxigenin antibodies, and direct incorporation of digoxigenin-11-dUTP into PCR products, followed by antibody detection. Results are most encouraging with the direct incorporation strategy, with recent experiments showing detection of a single copy of proviral DNA in approximately 85% of cultured cells, with low background. Optimization of this procedure is in progress. Other potential strategies being analyzed include the use of biotinylated PCR primers followed by streptavidin-fluorescein detection, and Bromodeoxyuridine triphosphate incorporation into PCR products, followed by antibody detection.

"Detection of HIV-1 Infected Cells by Flow Cytometry using Direct Detection Fluorescent *In Situ* Hybridization (FISH)"- ---

Protocol Description:

A sensitive Flow Cytometric technique was developed by this Program Area which enables the detection of HIV-1 infected cells actively expressing viral mRNA and the ability to distinguish the phenotype of these cells. This technique requires labeling HIV-1 DNA with a nucleoside analog containing digoxigenin, then hybridizing the labeled probe to fixed, permeabilized HIV-1 infected or uninfected cells, followed by detection of the hybridized probe using a fluorescent labeled, anti-digoxigenin monoclonal antibody. The Flow Cytometer is used to quantitate and sort the fluorescently labeled HIV-1 infected cells hybridizing the labeled probe. This *in situ* hybridization technique can be used in conjunction with fluorescently labeled antibodies directed against cell surface markers to identify the phenotype of the infected cells in PBMC or lymph nodes.

Specific objectives include:

- a. Develop Fluorescent *In Situ* Hybridization (FISH) and Flow Cytometry as a method of rapidly quantitating the number of HIV infected cells actively producing mRNA in:
 - patient whole blood
 - patient tissue cell suspensions
 - frozen patient samples
- b. Develop an approach to identify the phenotype of the HIV-1 infected cells by employing Flow Cytometry FISH analysis and Fluorescent Activated Cell sorting (FACs) simultaneously.
- c. Utilize both Flow Cytometry FISH analysis and FACs to quantitate the number of infected cells expressing viral mRNA's and to identify the phenotype of the infected cell during drug or gene therapy.

Significant Findings:

- Developed fixation and cell permeablization procedures for FISH. Developed unique cell staining procedure (Evans Blue) to detect positive hybridization.
- Directly correlated flow cytometry FISH analysis of cell cultures with the standard slide method of *in situ* hybridization using alkaline phosphatase and NBT/BCIP.
- Demonstrated with Cell culture mixing experiments that flow cytometry FISH analysis could detect 1 infected cell in a population of 1000 uninfected cells.
- Successfully used Flow Cytometry FISH assay to quantitate the number of cells expressing HIV mRNA in a population of newly infected PHA stimulated PBMC's
- Performed combination Flow Cytometry FISH assay and FACs analysis. Demonstrated that the CD4+ sorted cell population in PHA stimulated PBMC's had the greatest number of cells actively expressing viral mRNA.
- Showed that newly infected PHA stimulated PBMC's did not demonstrate any increases in mRNA signal by either increasing the viral contact time or increased culture time.
- Pursued indirect detection methods. This assay can be developed following the successful completion of the procedure.

"Detection of HIV-Infected Cells using Flow Cytometry, *In Situ* PCR, and Fluorescent *In Situ* Hybridization"-

Protocol Description:

Ashley Haase demonstrated that levels of HIV mRNA and DNA in cells can be monitored by using fluorescent labeled probes after *in situ* PCR (Science, Vol. 259). In collaboration with the Cellular Pathology Laboratory work unit, the researchers are currently developing a similar approach in identifying infected cells containing HIV DNA and infected cells actively expressing HIV RNA in PBMC and in tissue compartments such as the lymph node. Using the same fixation and cell permeablization procedures described before, PCR primers can be used to amplify genomic or subgenomic mRNA and/or HIV DNA. The PCR product will be detected by hybridization with specific fluorescent probes.

Specific objectives included:

- a. Develop *in situ* PCR, Fluorescent *In Situ* Hybridization (FISH) and Flow Cytometry as a method of rapidly quantitating the number of HIV infected cells and the levels of viral mRNA in:
 - patient whole blood
 - patient tissue cell suspensions
 - frozen patient samples.
- b. Develop an approach to identify the phenotype of the HIV-1 infected cells by employing Flow Cytometry Fluorescent *In Situ* Hybridization (FISH) analysis and Fluorescent Activated Cell sorting (FACs) simultaneously.
- c. Utilize both Flow Cytometry FISH analysis and FACs to identify the phenotype of the infected cells and the cells expressing viral RNA during drug or gene therapy.

Significant Findings:

- The cell fixation and permeabilization parameters for *in situ* PCR are currently being assessed. Conditions must be established that ensure the cellular retention of the PCR products and hybridization probes.
 - Pilot studies on uninfected cells, A301, and chronically infected cells, ACH2, revealed that current procedural conditions show high levels of PCR products *in situ* in the infected cells, low levels in uninfected cells with minimal leakage of PCR products in the supernatants.
 - Cell recovery experiments determined the best method for both PCR and retention of light scatter characteristics.
 - Successful digoxigenin probes hybridized to gamma actin amplimers showed fluorescent intensities greater than 90% as compared with controls.
 - More rapid direct measurements were developed from indirect probe detection to detection systems using fluorochromes or combination with fluorochromes within amplimers. Current studies show increases in gag gene products using dUTP-DIG incorporation during PCR. Detection of these products on the flow cytometer showed greater than 80% ACH2 cells were positive over controls (<0.1%).
-

"Viral Burden - RNA and DNA PCR Analysis in Purified Sorted Cell Populations"-

Protocol Description:

CD4 cell populations with different cell surface determinants appear to be infected at different rates during the course of disease (AIDS Research and Human Retroviruses, Vol.7,1991). If this is the case, then it might be possible to measure the changes in infection in the different CD4 cell populations and use the data generated as a surrogate marker for disease progression or therapeutic intervention. In collaboration with the Intervention Assessment Program Area, the Flow Cytometer is being utilized to sort the different CD4 cell populations and to determine the viral burden in these cells by RNA and DNA PCR. A quantitative plate assay for PCR is also being developed to screen multiple samples rapidly and efficiently.

Specific objectives include:

- a. Develop Fluorescent Activated Cell sorting using the Flow Cytometer to rapidly and efficiently sort patient PBMC's and patient tissue cell suspensions in the various CD4+ cell populations.
- b. Develop RNA and DNA PCR plate assay with material obtained from fixed cell sorts.
- c. Validate this assay with patient material on drug or gene therapies.

Significant Findings:

- The researchers were able to highly purify fixed CD4+ cells with contamination rates of less than 0.01%.
- Stained uninfected cells (Leuko-PAK) were successfully sorted and found to be negative by PCR amplification when mixed with a HIV-1 chronically infected cell line (ACH2).
- Quality controls for PCR amplification were developed using DNA derived from fixed infected ACH2 and uninfected cells and SK primers.
- Results from pilot studies showed that HIV infection in CD4+ cells increased with Walter Reed staging.
- Preliminary experiments indicated that the SRA plate assay (Hybridization Protection Assay or HPA) system for the detection of PCR products was useful in determining positive cell endpoints.

- A statistical model designed to measure the cellular viral load at 50% indicated a range of values from 11 to 800 infected CD4 cells.

Gene Therapy Work Unit

The primary mission of this unit is to develop an efficient gene delivery system capable of transducing and expressing HIV immunogens to induce immune responses in uninfected personnel or augment immune responses in HIV infected personnel and anti-HIV antiviral agents to inhibit virus replication and preserve the immune system of HIV infected personnel. The following are the primary objectives of this program:

- Develop efficient gene delivery systems capable of transducing and expressing genes encoding HIV and SIV antigens and anti- HIV/- SIV antivirals.
- Evaluate efficacy, safety and toxicity of prophylactic and immunotherapeutic vaccine and antiviral strategies using gene delivery systems in mouse, SCID-Hu mouse and Rhesus macaque models.
- Evaluate clinical efficacy of the gene therapy strategies in both early stage HIV infected and uninfected military populations.

To accomplish these objectives, the following projects are ongoing or proposed. Details of each are described below as well as significant findings or results.

Technical Approach:

"Molecular and Genetic Characterization of HIV-1-Based Packaging Cell Lines and Recombinant Retroviral Vectors"-

Project Description :

The researchers are developing an efficient gene delivery system capable of transducing and expressing HIV immunogens and anti-HIV antiviral agents to prevent HIV infection in uninfected individuals or to preserve the immune system of HIV-infected populations.

Significant Findings:

This program area has developed and characterized a prototype HIV-based stable packaging cell line that when transfected with an HIV-based

retroviral vector, produces packaged vectors that deliver marker genes to susceptible CD4+ cells. Although the recombinant vector titers are low, the vectors stably integrate into the target CD4+ cell genome, and no structural rearrangements have been detected. While no full length HIV promoter-directed transcripts were observed, a low level of vector-specific transcription consistent in size with spliced transcripts arising from the HIV promoter was detected. High level expression of full length vector transcripts requires the HIV-1 regulatory gene products Tat and Rev. This requirement for HIV regulatory gene products suggests that HIV-based vectors can express immunogens or antivirals either constitutively (using the internal promoter present in the vectors), or inducibly, in direct response to HIV infection. Furthermore, no replication competent virus has been detected in any transducing particle preparations to date. A manuscript describing these results has been published recently by a Program Area researcher.

Ongoing research focuses on increasing the titer of packaged vectors produced by the stable HIV-based packaging cell lines. Preliminary results suggest that nonadherent lymphocytic packaging cell lines (as opposed to the prototype adherent packaging cell line) produce higher titer vectors. Therefore, vector production in a wide variety of adherent and nonadherent stable packaging cell lines is being measured. Concurrently, transcriptional, translational, and post-translational events within these candidate packaging cell lines are being examined in order to identify defects responsible for the low titer of recombinant vectors observed in the prototype packaging cell line. The information obtained from these studies will be used to generate packaging cell lines that produce higher titers of recombinant vectors, with a concurrent reduction in the potential for forming replication competent virus.

While the bulk of the research effort is directed at increasing vector titers, a parallel effort focuses on development of HIV vectors containing antiviral payloads. The first such vector, containing an inducible or a constitutively expressed Herpes Simplex thymidine kinase gene, has been constructed. Assay of the antiviral efficacy of this construct is ongoing.

"Prophylactic and Immunotherapeutic Vaccine Strategies : Direct DNA Transfer with T Cell Activation as a Novel Strategy for the Development of New HIV vaccines" -

Hypothesis:

To date, the immune mechanisms that might contribute to the prevention or reduction of the pathological effects of HIV infection in the military population are poorly understood. Early stage military personnel infected with HIV show potent T cell proliferative and cytotoxic T cell responses specific to HIV. Moreover, these individuals have developed high titer anti-HIV antibody responses capable of neutralizing cell free viral transmission in culture. Despite the apparent potency of these responses, the immune mechanisms induced in infected individuals are not sufficient to clear the virus and thus prevent disease progression. However, the immune response induced in HIV infected personnel may significantly contribute to controlling infection before manifestation of AIDS. This program area is focused on the development of an expression and delivery system capable of expressing all or some of the HIV and SIV antigens in transduced cells *in vivo*, in the absence of virus replication. The product being developed by this program must be an effective means of activating virus specific Class I and Class II restricted immune responses. The product being developed is a simple immunization strategy using direct intramuscular injection of plasmid DNA expression vectors containing specific viral genes. A mouse model is being developed to evaluate the potency and specificity of the immune responses induced by this approach. Mechanisms for improving DNA delivery, such as liposome delivery systems, and augmenting immune responses, such as co-injecting co-stimulatory molecules or cytokines, will also be evaluated. Additionally, the product must be safe, nontoxic and be effective after one or perhaps two administrations.

Project Description:

Protective immunity to viral infections might be developed by simple intramuscular injection of "naked" plasmid DNA expression vectors containing specific viral genes. Strategies to develop and enhance this method of vaccination for HIV are being developed and evaluated. Specific objectives include:

- a. Develop a murine model of vaccination through direct DNA transfer.
- b. Generate autologous CTL targets expressing HIV and SIV antigens and appropriate controls.
- c. Generate plasmid expression vectors capable of any viral gene of interest.

- d. Generate plasmid expression vectors capable of co-expressing the costimulatory molecules such as B7.1 and B7.2 and the viral gene of interest.
- e. Evaluate the immune responses in mouse model following direct DNA transfer.
- f. Develop tumor model system to evaluate efficacy of immune response.
- g. Determine strategies to augment the immune response to direct DNA vaccination.
- h. Apply these results to nonhuman primate/SIV studies with the goal of application in human trials.

Significant Findings:

- Vaccinating plasmids capable of coexpressing the murine B7-1 gene have been constructed. These plasmids are able to drive high level expression of mB6-1 when transfected into COS7 cells.
- Vaccinating plasmids capable of co-expressing the murine B7-2 gene are under construction and are near completion.
- The HIV IIIB env gene and the SIV env gene have been cloned into mB7-1 co-expressing plasmids. Sufficient amounts of these plasmids have been grown up in culture for vaccination.
- ELISA assays for anti-gp120 and gp-160 antibodies have been established at the Naval Medical Research Institute. Baseline studies establishing the kinetics of antibody responses to recombinant gp120 protein immunization have been completed.
- Retrovirally transduced env-expressing autologous 3T12-3 Balb/c fibroblast cell lines (H-2b) and appropriate controls have been established for use as target cells in CTL assays. Optimization of CTL assays using these cells is currently underway.
- *In situ* histochemical staining of muscle tissue at the site of DNA infection demonstrates that B7-1 and B7-2 expression on myocytes is not detected.
- DNA vaccination of groups of mice with vector alone, env and env/B7-1 expressing vectors have just been initiated.

Future Studies Planned:

The ultimate implementation of this vaccine strategy will be dictated by the findings in these studies, and will be the subject of future applications. Products expected from this work unit include FDA-approved DNA vaccine constructs.

"Assessing Anti-HIV Antiviral Gene Therapy Strategies in Mature CD4+ T-Cells Susceptible to HIV Expression and Replication"-

Project Description:

This project explores mechanisms used by HIV to create tailored expression of therapeutic agents which are designed to inhibit HIV expression and replication. By arming HIV-susceptible cells with effective antiviral genetic constructions, the researchers expect to stalemate HIV dissemination in HIV-infected individuals. By maintaining normal cellular function in uninfected cells while suppressing HIV expression in cells harboring HIV, infected individuals should continue to live in an early-stage, asymptomatic state, allowing them to continue their life in spite of HIV. Specific research objectives include:

- a. Efficiently deliver anti-HIV antivirals to HIV-susceptible cells using retroviral vectors.
- b. Assess antiviral activity of various anti-HIV genetic constructions in tissue-cultured cells and primary cells.
- c. Test promising anti-HIV antiviral strategy in an animal model system and in early-stage HIV-infected individuals.

Significant Findings:

A number of anti-HIV genetic constructions were made within both MuLV- and HIV-based vectors. These included antisense (pJM357B@RRE, pJM473, and pJM475), ribozymes/gag transdominant peptide (pJM354, pJM321neoA), TAR decoys (pJM480neo), rev transdominants (pJM479neo, pJM478), and alpha2-interferon (pBK89, pJM362A&B, pJM363, pJM456, pJM458, pJM474). A 96-well format evaluation for antiviral assessment of genetic constructions was developed, which allowed the researchers to quickly test and compare our various antiviral strategies against laboratory and clinical isolates. To date not all of the above constructions have been tested, however, all have been molecularly constructed, ready for packaging as MuLV or HIV vectors. At the present time, the alpha2-interferon constructions demonstrated antiviral activity approaching complete inhibition of p24 activity in 7 day microtiter

assays using 100 TCID₅₀ of input virus. In addition the researchers are evaluating various anti-HIV genetic constructions within molecular clones of HIV. The antivirals replaced that portion of nef not overlapping the 3' LTR and are capable of producing replication-competent HIV. These included proIFN (pJM485A&B), proTAR-3 (pJM484A&B), and proM10 (pJM453).

To date, all testing of antiviral genetic constructions has been evaluated in transformed T-cell lines. Strategies are being developed which should allow us to evaluate our anti-HIV genetic constructions in primary PBMCs. One such strategy will be to replace the neomycin selectable marker with a mouse cell surface marker. The mouse Ly6A.2 gene will be used for this purpose.

"Assessing Anti-HIV Antiviral Strategies in Pluripotent Hematopoietic Stem Cells"

Project Description:

This project exploits the proliferation and differentiation potential of hematopoietic stem cells and their progenitors. The introduction of genetic material into one stem cell is equivalent to targeting thousands of mature monocytes and lymphocytes. Once armed with expression systems where anti-HIV genetic products are made in response to HIV infection, these mature cells will limit further HIV dissemination and will provide a repertoire of functional mature blood cells to sustain an HIV-infected individual.

Specific research objectives include:

- a. Efficiently deliver anti-HIV antivirals to primitive hematopoietic stem cells.
- b. Assess antiviral activity of various anti-HIV genetic constructions in retroviral vectors in tissue culture stem cell equivalence and in mature CD4+ cells derived from differentiated primary CD34 cells.
- c. Test promising anti-HIV antiviral strategy in an animal model system and in early-stage HIV-infected individuals.

Significant Findings:

Two publications were submitted by this program area pertaining to the cell type susceptible to HIV infection within the human bone marrow compartment. One publication cited the use of the clonal human stromal cell

line, Lof(11-10), to study the effect HIV might have on the ability of these cells to support bone marrow-derived CD34+ progenitor cells in culture. The researchers found that the Lof(11-10) cells were not infectible by HIV, so molecular clones of HIV were introduced into these cells by transfection. There was no qualitative difference in the levels of cytokine production between HIV-expressing and control Lof(11-10) cells. Furthermore, conditioned media derived from HIV-expressing and control Lof(11-10) cells added to CD34+ progenitor cells yielded similar colony formation in methylcellulose assays. This study concluded that stromal cells within the bone marrow compartment, such as Lof(11-10) cells, are capable of both normal cytokine production and supporting hematopoiesis in spite of HIV expression.

The second publication revisited the question of whether CD34+ stem cells are susceptible to HIV infection. In the presence of human growth factors, CD34+ cells differentiated towards the myeloid series into monocyte/macrophages and granulocytes. However, because CD34+ cells rapidly differentiate in culture, the possibility exists that infection of CD34+ progeny cells may be responsible for the positive HIV signal observed in these cultures. To address this possibility, purified CD34+ cells were infected with HIV-1-Ba-L and then reselected either before or after exposure to HIV. When double-purified CD34+ cells were infected with HIV, a positive HIV signal was observed, but when reselection was done after HIV exposure, the CD34+ cells were negative for the presence of HIV. Our data suggested that the differentiated progeny cells and not the CD34+ cells were infected with HIV and responsible for the positive HIV signal observed in the cultures. The studies concluded that human CD34+ stem cells are not infectable with HIV-1-Ba-L, agreeing with results found in HIV-infected patients.

Both of the above studies are developing the foundation necessary to accomplish our research objectives. The characterization of the Lof(11-10) cells as a human cytokine cell line, expressing IL-1alpha, IL-1beta, IL-6, IL-8, M-CSF, G-CSF, GM-CSF, TGFalpha, and stem cell factor, led to the use of this cell line to supply the human cytokines needed for the growth and differentiation of CD34+ cells to reconstitute the SCID mouse. The study demonstrating that CD34+ cells are not susceptible to HIV infection demonstrated that our HIV-vector based gene delivery system can not be used to target the pluripotent CD34+ stem cell. This result has led the researchers to examine other delivery systems for stem cell targeting. Presently, an AAV vector containing alkaline phosphatase is being sent to us by Dr. Terwilliger (New England Deaconess Hospital, Boston) and we are sending an SCMV-alkaline phosphatase-expressing vector with the LOX recombination sequence to Dr. Glorioso (U. of Pittsburgh) for the construction of an HSV-1-based defective gene delivery virus. These delivery systems together with a MuLV-based vector containing the alkaline phosphatase gene, made in our own laboratory, will be tested using identical procedures developed in our HIV and CD34+ cell publication.

"Culture Systems for the *In vitro* Expansion of Primitive Hematopoietic Bone Marrow Cells and CD4+ Cells"-

Project Description:

Efficient and novel culture systems will be developed for the *in vitro* expansion of CD4+ T cells and human hematopoietic stem cells. These cell growth systems will be used for treatment of HIV-infected patients through adoptive immunotherapy strategies, retroviral vector-mediated gene transduction of hematopoietic progenitor cells and T cells. These systems will also be utilized in the laboratory as a source of CD4+ T cells and CD34+ hematopoietic cells for gene transfer, and laboratory-based patient monitoring.

- I. CD4+ T cell expansion
- II. CD34+ cell expansion

Research Hypothesis:

Current strategies contemplated for therapy and monitoring of patients with HIV infection require the *in vitro* propagation of cells derived from human bone marrow. For example, the monitoring of sensitivity to chemotherapy of HIV isolates requires a convenient source of large numbers of CD4+ T cells. Similarly, adoptive immunotherapy with CD4+ T cells requires expansion of the patient's T lymphocytes. Finally, gene insertion into T cells or hematopoietic stem cells may provide direct and curative treatment for acquired immunodeficiency disease. At this point, however, the major obstacles for gene therapy into primary human bone marrow stem cells are the small number of these primitive stem cells, the quiescent nature of these cells and the lack of a defined *in vitro* culture system to support the proliferation, self-renewal and expansion of these cells. There are similar limitations in the *in vitro* production of CD4+ T cells, as current technology results in the *in vitro* expansion of T cells displaying the CD8+ phenotype, with a progressive loss of CD4+ cells during T cell expansion.

Therefore, the central hypothesis of this proposal is that current technology for the *in vitro* propagation of bone marrow derived cells is inadequate, and that this limits the development of improved therapy of patients with HIV infection. It is our hypothesis that expansion of human CD4+ T cells by recruiting quiescent cells into active proliferation. Furthermore, it is predicted that the *in vitro* endothelial cell microenvironment hematopoietic culture system will also increase the efficiency of gene transfer into the most primitive hematopoietic progenitor cells and expand these transfected stem cells to clinically useful levels.

Goals:

1. Establish a system for the large-scale expansion of primary human CD4+ T cells for use in monitoring drug resistance in chemotherapy trials (standardized T lymphocyte cell bank), adoptive immunotherapy of HIV-infected patients and gene therapy of primary T cells.
2. Establish a system for the culture and expansion of primitive human primary hematopoietic stem cells for use in gene therapy of stem cells in HIV infection.

Objectives: I. CD4 expansion

- a. Expansion of CD4 T cells by co-stimulation with antibodies to CD28 and CDC. Evaluate T cell identity, function and polyclonality. Validate scale-up approach.
- b. Perform 4 to 6 log *in vitro* expansion of CD4 T cells derived from HIV infected patients by co-stimulation with antibodies to CD28 and CD3 in the presence of ddI, nevirapine and protease inhibitor to suppress possible virus replication.
- c. Evaluate T cell identity, function and polyclonality.
- d. Prepare for autologous adoptive immunotherapy trial of AZT naive HIV infected patients with CD4 levels of 500.
- e. Develop approaches to improve on efficiency of retroviral transduction in these expanded cells. Evaluate T cell identity, function and polyclonality after transduction.
- f. Determine if expanded CD4+ T cells maintain the capacity to be infected with HIV. If so expand CD4+ T cells for cryopreservation and subsequent use in *in vitro* drug resistance assays.

Objectives: II. CD34 expansion

- a. Determine the culture conditions for optimal human stem cell proliferation, expansion, and self-renewal. Investigate the effects of various hematopoietic growth factors in this culture system on gene transfer into primitive hematopoietic stem cells (CD34+).
- b. Determine whether transfected hematopoietic stem cells in this culture system maintain their capacity to differentiate into both myeloid and lymphoid lineages.

- c. Develop approaches to improve on efficiency of retroviral transduction in these expanded cells. Determine if transduction affects self renewal capacity and differentiation capacity of these cells.
- d. Develop methodologies for large-scale stem cell expansion and gene transduced/transfection into primitive hematopoietic progenitor stem cells.

Significant Findings:

- The researchers determined that CD28 and CD3 co-stimulation permits the selective expansion of CD4+ T cells. Currently 1×10^6 fold expansion over 4 to 6 weeks of culture has been obtained. Culture systems that use lectins and IL-2 for T cell propagation yield a lower expansion of cells, and the cells are of the CD8+ phenotype.
- The researchers are evaluating two approaches for maintaining and scaling up lymphocyte expansion from tissue culture flasks into systems that will allow culture of up to 3×10^{10} cells. The first of these systems is a hollow fiber apparatus manufactured by Cellco. The second is a gas permeable tissue culture bag manufactured by Baxter. This system is employed by Dr. Steven Rosenberg's laboratory to expand Tumor Infiltrating Lymphocytes (TILs).
- Results established that CD4+ T cells from HIV-infected patients can be expanded 0.2 - 12×10^4 fold and cleared of virus (as determined by p24 levels and PCR) when stimulated with anti-CD3 and anti-CD28 antibodies in the presence of AZT + ddI + pyridone L-697,66A.
- An adoptive immunotherapy protocol in which autologous expanded CD4 cells are reinfused into HIV infected patients is currently under scientific review.
- A unique *in vitro* endothelial microenvironment culture system (PMVEC) was developed which supports the amplification of human and nonhuman primate hematopoietic progenitor cells (CD34+ CD38- bone marrow cells). Utilizing this system, a 10,000-fold increase was achieved in CD34+ stem cells and an overall expansion of mononuclear cells of $> 1 \times 10^6$ -fold over 35 days in culture.

- This hematopoietic culture system was adapted to large-scale hollow fiber bioreactor configurations. Starting with a 15 ml bone marrow aspirate, enough cells can be grown in these bioreactors in 10-14 days to transplant into a patient.
- The researchers demonstrated that human CD34+ stem cells are not susceptible to HIV infection when grown in the PMVEC culture system.
- Experiments are underway examining the efficiency of gene transfer into CD34+ cells grown in the PMVEC culture system. Preliminary data suggests that CD34+ cells can be transduced in this system.

There are five patent applications pending that protect the technology developed at NMRI for the expansion of CD4+ T cells and CD34+ hematopoietic stem cells:

1. "CD28 pathway immunoregulation" Navy Case #74,610; filed 4/7/92.
2. "Enhancement of CD28-related immune response" Navy Case #74,611; filed 4/7/92.
3. "Immunotherapy involving stimulation of THCD28 lymphokine production: Navy Case #74,652; filed 4/7/92.
4. "Ex vivo culture system for the rapid expansion of primitive hematopoietic CD34+ progenitor cells: Navy Case 75,259; filed 10/28/93.
5. "Large-scale amplification of human hematopoietic progenitor cells in hollow fiber bioreactor culture system" filed 1/21/94.

Future Plans:

The ultimate implementation of these technologies will be dictated by the findings in these studies, and will be the subject of future applications. Products expected from this work unit include FDA-approved technology for the propagation of hematopoietic progenitor cells and CD4+ T cells.

IV. CONCLUDING REMARKS

The HIV Disease Prevention Program Area remains a national leader in technology development as well as in clinical trial design and execution. A focus on the development and validation of virologic and immunologic surrogate markers for evaluation of interventions in patients has resulted in the development of several assays, in collaboration with other military investigators, the ACTG Virology Committee and the SRA. Technologies developed include:

- Microtiter-based drug susceptibility assay
- PCR detection of RT codon 215 (ZDV resistance)
- Microtiter plate based SI/NSI assay
- T cell activation assay (NMRI)
- Sequencing of PCR products for drug resistance mutations
- Library of drug-resistant HIV RT genes

Within the area of development of novel combinations of chemotherapeutic agents to prevent disease progression, this Program Area also has effectively utilized clinical samples obtained during clinical trials to identify and validate a panel of virologic and immunologic surrogate markers for use in other chemotherapy trials. In one study the systematic collation of clinical data and specimens from patients on prolonged antiretroviral therapy has proved an invaluable resource for drug resistant studies. This Program Area has also utilized the military HIV screening program to monitor the HIV epidemic in real time through studies of newly detected seroconverters. The first AZT resistant seroconversion was documented by the Program Area in collaboration with the University of Minnesota; this Program Area also demonstrated that AZT resistant HIV-1 seroconversion (215 mutation detected by PCR) increased from 3% in 1988-1991 to 19% in 1993-1994 in the U.S. and Switzerland.

In the area of Gene Therapy, this Program Area has developed supporting technologies to include:

- HIV Packaging Cell Line
- Retroviral Vectors
- Antiviral Gene Constructs
- Naked DNA Immunization Vector
- Plasmid-derived Attenuated SIV/HIV
- Target cells for CTL assays.

In addition to these technologies, this Program Area has worked closely with NMRI in the design and development of the first Phase I Gene Therapy protocol. There are five patent applications pending that protect the technology developed at Naval Medical Research Institute for the expansion of CD4+ T cells and CD34+ hematopoietic stem cells.

The cellular pathology unit has also developed new technologies to assess the impact of HIV disease progression. These technologies included:

- Immunohistochemistry
- *In Situ* hybridization - radioactive/fluorescent
- Image Analysis
- Double Label Methods (combination immunohistochemistry/*in situ*)

As evidenced by the technologies engineered and accomplishments noted above, this program area represents a well-balanced scientific program of clinical and laboratory projects involving extensive collaborations within the HIV Research Program and also with outside military investigators as well as other federal agencies and private industry. As a measure of research productivity, this Program Area authored or coauthored over 50 publications and presentations during this 12 month reporting period. With access to early stage patients, invaluable longitudinal clinical specimens and the proven ability to develop new technologies, this program area remains a leader in the effort to understand and eradicate HIV.

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IMMUNOREGULATION

USAMRMC Program Area Coordinator: rgp160 Program Manager:

LTC Deborah Birx, MD, MC, US Army Keith Johnson

Program Area Summary:

The overall goal of the Immunoregulation Program Area is the development, evaluation and demonstration of efficacy of HIV specific vaccines for both treatment and potential prevention of HIV infection and disease. Research within this Program Area utilizes clinical trials (*in vivo* models) and laboratory projects (*in vitro* models) in its pursuit of identification of effective HIV immunoregulatory mechanisms.

Human Use Protocols

Protocol Number	Title (Abbreviated)	Principal Investigator
RV21A	Recombinant gp160 Phase I	Redfield
RV21B	Recombinant gp160 Phase II	Redfield
RV21C	Civilian Sites gp160 Phase II	Redfield
RV51	gp120/Genentech	Redfield
RV57	gp160/AZT (completed)	Redfield

Laboratory Research Projects

Titles (abbreviated)	Principal Investigator
HIV-1 Cellular RNA Load and Splicing Patterns	Michael
Charact. of a Long-term Survivor of HIV-1 Infection	Michael
Broadness of Seroreactivity to the V3 loop of HIV-1	Michael
Naturally Occurring Genotypes of the HIV-1 Long Terminal	Michael
Negative-Strand RNA Transcripts are Produced in HIV-1 Infected Cells	Michael
HIV-1 Proviral Genotypes from the PBMCs of an Infected Patient	Michael

Correlation between Monoclonal Antibody Kinetic Constants	Van Cott
Development of a Serotyping Assay to Differentiate Individuals (B or E)	Van Cott
Dependence of gp120 Conformational Structure on Binding to Epitope-specific Monoclonal and Polyclonal Antibodies	Van Cott
Comparisons between the Immune Responses Elicited by Candidate HIV-1 gp120/160	Van Cott
Determine the Role HIV-1 Serum V3 Antibodies Play in Neutralizing Primary HIV-1 Isolates	Van Cott
Characterization of a Soluble Secreted Oligomeric gp160 Molecule	Van Cott
Phenotypic and Genotypic Analysis of Virus Isolates from HIV-1 Infected Children with Rapid vs. Slow Progression	Robb
Comparative Susceptibility to Serum Neutralization of Viral Isolates Derived from LN, Plasma and Peripheral Blood	Robb
Mechanism and Therapeutic Implications of Transcriptional Competition between Isolates	Kim
Cytokine Enhancement of Anti-HIV Immune Responses	Kim
Mapping the T-Lymphocyte Epitopes in gp120	Sitz/Ratto
Characterization of Phenotype Changes	Sitz
Evaluating Crossreactive T-lymphocyte Epitopes within gp120	Sitz/Ratto
<i>In vivo</i> T-lymphocyte Mapping	Sitz
RV 21 Naive vs. Memory Phenotyping	Sitz
RV 51 International Serotyping	Sitz
RV21II b and c Phenotyping	Sitz
Establishment and Evaluation of CD4+ T-Lymphocyte Lines	Ratto
CD4+ Specific T-Lymphocyte Lines Developed from 2 HIV Patients Recognize Different Epitopes on the V3 loop	Ratto
Characterization of Recombinant and Purified gp120 prep.	Van Cott
Mapping CD4 T-Lymphocyte Epitopes in a Long Term Survivor	Ratto
Mucosal Immunity to HIV-1	Burnett
Cross-Reactivity between Clades of Serum from HIV infected Individuals or Envelope Immunized Rabbits	Loomis

Microencapsulation of HIV-1 Envelop Glycoprotein	Burnett
Antibody Purification	Loomis
Serology	Loomis
PEPSCAN	Loomis
Spectrotyping	Loomis
SIV Serology	Loomis
Peptide Mapping Strategies	Loomis

I. OVERVIEW

The overall objective of the Immunoregulation Program Area is the identification of effective HIV immunoregulatory mechanisms *in vitro* in the laboratory and *in vivo* through clinical research. The identification of these immune mechanisms is critical for successful HIV vaccine development for prevention. Research in this Program Area is centered around the scientific investigation of human HIV immunity (with emphasis on cellular immune mechanisms) and the mechanics of HIV replication in the human host. The viral expression *in vivo* and the characterization of the dynamic interaction between immune mechanisms and viral expression is shedding light on human HIV immunoregulation.

The scientific strategy of this Program Area is encompassing yet highly focused. The plan is one of interdependent, concurrent and basic science investigations combined with applied clinical evaluations of candidate vaccines and immune-based products. An extensive clinical research component is essential for the *in vivo* demonstration of the *in vitro* findings. The ongoing vaccine trials which comprise the clinical research component have provided the laboratory research unit with invaluable samples of serum and cells that are essential for applied laboratory based research. The Immunoregulation Program Area has become increasingly involved in new product development on the preclinical side and is providing the immunologic support for the Preventive Vaccines Program Area activities.

During the past 12 months, the large DoD Phase II double blind placebo controlled trial entitled "Active Immunization of Early Patients with Recombinant gp160 HIV protein: Phase II Study of Toxicity, Immunotherapy, *In vivo* Immunoregulation and Clinical Efficacy" has undergone a transition. Wyeth-Ayerst, a corporate sponsor with MicroGeneSys for the rgp160 vaccine, withdrew from the trial for financial reasons. This left a substantial void relative to the trial execution, including trial monitoring, data base platform, contractual relationships to the civilian sites, and senior medical monitoring. In response to this transition, the Foundation appointed a gp160 Program Executive Officer to provide programmatic oversight and coordination of this multi-center trial (6 civilian sites, 7 NIH sites and 3 DoD sites). A strategic plan was rapidly developed and implemented to meet all the trial requirements. This plan utilized a centralized approach to the monitoring, provided for the design of a functional data base and streamlined and identified critical needs within the trial execution. A skilled monitoring group was trained and is working with the Foundation MIS group in the development of the data base for the trial. Contractual relationships have been executed with the civilian sites. The Deputy MMCARR Director was appointed senior medical monitor and with the Chief of Infectious Disease Services Dr. Charles Oster provide the medical oversight until trial completion. This integrated and independent approach to DoD trials will provide the template for all future DoD trials within the Division of Retrovirology.

II. RESEARCH GOALS AND OBJECTIVES

The overall objective of the Immunoregulation Program Area is the identification of effective HIV immunoregulatory mechanisms *in vitro* in the laboratory and *in vivo* through clinical research. This Program Area has refocused its effort on the following objectives:

- A. Develop and apply new technology to measure and characterize HIV specific immune responses;
- B. Develop and apply new technology to measure and characterize HIV load and expression *in vivo*;
- C. Define mechanisms responsible for effective HIV immunoregulation;
- D. Develop the required technology and scientific infrastructure to define the protective immune response and aid in the successful execution of preventive vaccine trials.
- E. Assist in the development and evaluation of candidate HIV vaccines in a stepwise fashion for efficacy in the prevention of HIV infection.

III. TECHNICAL APPROACH

The Immunoregulation Program Area achieves its objectives through the conduct of laboratory projects and the execution of Phase I and Phase II Vaccine trials. These projects and protocols are described below; significant results are also given.

Clinical Trials

RV21A-"Active Immunization of HIV Infected Patients with Recombinant gp160 HIV Protein: Phase I Study of Immunotherapy, Immunogenicity and Toxicity"-

Protocol Description:

The initial protocol was designed to evaluate the feasibility of post HIV infection vaccination with HIV viral products utilizing a recombinant rgp160 vaccine. This Phase I safety and immunogenicity trial began in April, 1989, was completed in November 1990 and was published in the New England Journal of Medicine in June 1991. A continuation trial was designed to assess the long term immunogenicity and safety of this product. The continuation trial began in November 1990, was modified by addendum in May 1992 and again in May of 1993.

Technical Approach:

During the primary extended immunization series, original trial responders were vaccinated every 4 months (160ug) and nonresponders at Day 0, 7, 30, 60, 90, and 120 (160ug) and then every 4 months (160ug). In March 1992 an addendum was approved to vaccinate volunteers every month for 12 months then every 2 months thereafter. Alteration in cellular and humoral immune responses to HIV specific proteins and changes *in vivo* and *in vitro* cellular immune function continue to be assessed.

Significant Findings:

26 volunteers re-enrolled in a continuation trial modifying the vaccination schedule to every other month. Two patients died on trial - one in an automobile accident and one of advanced HIV disease post discontinuation on trial. Three volunteers were discontinued. Four volunteers developed CD4 counts less than 200. Eight volunteers are receiving antiretroviral therapy (AZT/DDI). Long term safety and immunogenicity analysis is in progress. No evidence of *in vitro* or *in vivo* trial associated immune dysfunction has been demonstrated. No evidence of increased *in vivo* HIV expression has been demonstrated.

Number of Patients Enrolled: 26

Site: Walter Reed Army Medical Center (WRAMC), Washington, D.C.

RV21B-"Active Immunization of Early Patients with Recombinant gp160 HIV Protein: Phase II Study of Toxicity, Immunogenicity, *in vivo* Immunoregulation and Clinical Efficacy" -

Protocol Description:

This protocol is a Phase II, multi-center, double blinded, placebo controlled trial designed to evaluate the clinical efficacy by surrogate markers of post infection vaccination with rgp160 in the treatment of HIV infection and to validate adaptive anti-HIV immune responses in terms of *in vivo* HIV expression and clinical progression. Enrollment began in 11/90 and was initially limited to 140 volunteers. Following confirmation of safety and immunogenicity within this initial group, complete enrollment (608 volunteers) was approved spring 1992 and enrollment was completed on schedule November 1992. Initial efficacy analysis by surrogate markers was performed in December 1993 and final analysis is scheduled for the end of 1995.

Technical Approach:

300 DoD patients and 300 civilian patients were equally randomized to placebo or vaccine. All volunteers received intramuscular injections of 160ug of rgp160 or placebo on days 0, 7, 30, 60, 120, 180 and then at 2 month intervals through the completion of the trial. Changes in cellular and humoral immune responses, toxicity to rgp160, changes in CD4 counts, and shifts in viral burden have been studied.

Significant Findings:

Enrollment was completed and closed in November of 1992. Seventeen study sites are participating in this multi-centered trial. A total of 608 volunteers were randomized and 562 remain on trial. 8 volunteers have died and 38 volunteers have discontinued the trial. The protocol integrity continues to be maintained therefore minimal conclusions can be drawn at this time. The initial efficacy analysis was conducted in October 1993 in a blinded fashion and the Data Safety Monitoring Board recommended that the trial continue as planned. There have been 100 missed visits out of approximately 11,400 total visits. The missed visit rate continues below 1%; 99% of the visits are completed and 88% of the volunteers have never missed a visit.

Transition of Trial from Wyeth: Update

On June 16, 1994, Wyeth-Ayerst terminated its participation in the vaccine trial. Wyeth-Ayerst monitors conducted a close-out visit with each of the non-DoD 14 sites participating in the trial and the Foundation assumed responsibility for the trial as of 17 June 1994. The majority of the protocol execution issues

remained unchanged following the transition, with the exception of funding. Previously, Wyeth-Ayerst was directly responsible for funding six civilian clinical sites. Contract support for these sites will be provided by the Foundation, subject to negotiation with the individual sites.

Several issues were identified during the negotiation process as a result of the Foundation assuming responsibility for the trial execution. One site did not have an Affirmative Action Program. Wyeth-Ayerst had assumed responsibility for indemnification in case of injury resulting from participation in the trials. The question was raised about this responsibility now resting with the Foundation. Two civilian sites refused to comply with the patient registry requirement required by DoD for research volunteers. The sites felt that it was a breach of confidentiality for the patients. All issues have been resolved except for the patient registry requirement. This issue is awaiting resolution at USAMRMC.

Responsibility for data acquisition for the DoD sites is unchanged and remains with the Henry M. Jackson Foundation. The Foundation will be adding data acquisition for the civilian sites. All data entry will be centralized at the 13 Taft Court complex. The data analysis plan is the same, although a third member of the data analysis team will be provided by the Jackson Foundation instead of Wyeth-Ayerst. The other two members are Dr. Brundage, Chief, Preventive Medicine, WRAMC, and Dr. Mitch Gail, National Cancer Institute.

Dr. Charles Oster, Chief, Infectious Disease Service, WRAMC, will continue to serve as medical monitor for the military sites. Dr. Neal Boswell, Director, Laboratory Sciences, Division of Retrovirology, WRAIR, will serve as the medical monitor for the civilian sites. All clinical monitoring responsibilities have been assumed by the Jackson Foundation and will be centrally managed from the 13 Taft Court complex. An experienced Clinical Research Monitor has been hired to provide guidance to the clinical monitors, data management personnel, and protocol coordinators. In addition, this person will provide quality assurance review and ensure compliance with all FDA guidelines. Four clinical monitors have been trained by an outside firm and will complete two cycles of monitoring visits by the end of the calendar year. It is anticipated that the central management of the monitoring team will result in increased quality and uniformity in data collection.

Negotiations are currently underway for the transfer of the Wyeth-Ayerst data base to the Foundation. It is anticipated that this transition will be accomplished by the end of the calendar year. In addition, all administrative files will be forwarded to the Jackson Foundation from Wyeth-Ayerst.

MicroGeneSys will continue to hold the IND and will provide vaccine through trial completion. All reporting of adverse events to the FDA is still the responsibility of MicroGeneSys.

Patient Visits: 11,400

Site(s):

DOD

Walter Reed Army Medical Center (WRAMC), Washington, D.C.
National Naval Medical Center (NNMC), Bethesda, MD
Wilford Hall Medical Center (WHMC), San Antonio, TX

CIVILIAN

Maryland Biotechnology Center, Baltimore, MD
Infectious Disease Physicians, Annandale, VA
St. Joseph's Hospital, Tampa, FL
Graduate Hospital, Philadelphia, PA
Georgetown University, Washington, DC
St. Vincent's Hospital, New York, NY

NIH

Washington Regional AIDS Program, Washington, DC
Chicago Community Program for Clinical Research on AIDS, Chicago, IL
Delaware Community Program for Clinical Research on AIDS,
Wilmington, DE
AIDS Research Consortium of Atlanta, Atlanta, GA
Richmond AIDS Consortium, Richmond, VA

**RV51-"A Phase I Study of the Safety and Immunogenicity of IIIB rgp120/HIV
Vaccine in HIV-1 Seropositive Adult Volunteers"-**

Protocol Description:

The initial trial was a Phase I open label dose finding trial (100ug, 300ug, 600ug) in 19 volunteers followed by a blinded randomized component (300ug versus placebo) in 25 volunteers. The objective was to assess the safety and immunogenicity of the rgp120 vaccine in asymptomatic HIV-1 infected volunteers and compare the effectiveness of a 3 injection versus a 5 injection schedule and to compare the effect of variable dose level of the rgp120 vaccine.

Technical Approach:

Volunteers were vaccinated at 0, 1, 4, 8, and 16 weeks with follow up evaluations every 2 weeks through 24 weeks. This trial opened in November 1990 following confirmation of immunogenicity of 300ug and 600ug rgp120 dosage (8/92); a subsequently randomized arm enrollment opened September

1992 and closed December 1992. An extension addendum to evaluate variation in boosting schedule in terms of immunogenicity and safety began in April 1992 and was completed in September 1993. A protocol extension to administer 600ug every other month began in November of 1993.

Significant Findings:

Thirty-six volunteers continued this year in the extended immunization protocol. Six volunteers discontinued the trial this year, 3 for non-compliance and 3 who developed CD4 counts less than 200. Two volunteers are receiving antiretroviral therapy (AZT/DDI). The trial is scheduled for completion in November 1994 when a final analysis will be performed. No interim analyses were planned or completed beyond the first safety analysis performed at the end of the original immunization series.

RV 57-"Active Immunization of AZT Treated HIV Infected Patients with Recombinant gp160 HIV Protein: Phase I/II Study of Immunogenicity, Toxicity, and Effect on *In vivo* Immunoregulation"-

Protocol Description:

This is a Phase I multi-center, open label feasibility trial of rgp160 in patients with HIV infection (Walter Reed Stages 1-5) and currently receiving AZT. Specific objectives include:

- a. Assess the immunogenicity and safety of rgp160 in patients with more advanced HIV disease and in patients with early disease receiving AZT; and
- b. Determine parameters predictive of post infection immune responsiveness. Patients were stratified by T cell intervals. Each volunteer received 160ug of rgp160 on days 0, and 7 and at months 1, 2, 4, 6, 10. Trial duration was 12 months (not inclusive of preevaluation).

Technical Approach:

Volunteers initially were in Walter Reed Stages (1-5) to participate. They received AZT (at least 500mg/day for 12 weeks) stratified by baseline CD4 counts, and then 160ug intramuscular injections of this vaccine on days 0 and 7, and at months 1, 2, 4, 6, and 10. Safety parameters, adaptive anti-HIV immune responses, and parameters of HIV *in vivo* regulation were monitored.

Significant Findings:

This trial was completed in August, 1994. Toxicity was limited to local reactions; adverse reaction reports filed with Wyeth-Ayerst were all considered not vaccine related. Final analysis is pending.

Laboratory Projects

Technical Approach:

"HIV-1 Cellular RNA Load and Splicing Patterns Predict Disease Progression in a Longitudinally Studied Cohort"-

Project Description:

This project was a longitudinal study of RNA splicing patterns in 31 early-stage patients with an average follow-up time of 3 years.

Significant Findings:

Eighteen patients showed no evidence of disease progression whereas 13 patients showed either a $\geq 50\%$ reduction in baseline CD4 count or developed opportunistic infections. Levels of unspliced, tat, rev, and nef mRNAs in peripheral blood mononuclear cells were measured using a reverse transcriptase-quantitative, competitive, polymerase chain reaction (RT-qcPCR) assay. Viral RNA was detected in all patients at all time points. All 13 rapid progressors had ≥ 1 log greater viral RNA load than the slow progressors. In addition, 9 of the rapid progressors showed a greater than 3 fold reduction in the ratio of spliced to unspliced RNA over the 3 years of follow-up. Conversely, 2 slow progressors with intermediate levels of viral RNA showed no splicing shift. These results confirmed earlier observations that viral RNA is uniformly expressed in early-stage patients. The results further showed that cellular RNA viral load is predictive of disease progression. Importantly, the shift from a predominately spliced or regulatory viral mRNA pattern to a predominately unspliced pattern was both associated with disease progression and added predictive utility to measurement of either RNA class alone.

"Characterization of a Long-Term Survivor of HIV-1 Infection Lacking Cultivable Virus: Evidence for Defective Accessory Genes"-

Project Description:

This project focused on a patient who acquired HIV infection via transfusion 13 years ago and who has remained asymptomatic since that time. The blood donor and 2 other recipients of blood from the donor all developed AIDS. Despite a persistently positive Western blotting profile, the patient has been repeatedly culture negative with a very low viral burden. The patient's CD4 count has ranged between 385 and 600/ μ l with little change in lymphocyte subset percentages over the last four years. The researchers previously reported the lack of cultivatable virus from this patient and the presence of unique cellular immune responses to HIV-1 envelope glycoproteins.

Significant Findings:

The researchers now report the results of an intensive molecular genetic analysis of this patient over 6 years. Env gene sequences reveal an unusual Clade B quasispecies. The LTR/gag leader region sequences are wild-type and show normal basal and Tat-mediated transcriptional activities. Nef sequences from 1993 revealed 14 clones with open reading frames and 1 clone with an inactivating frameshift. The 14 open nef genes from 1993 were wild-type in both a cell surface CD4 downregulation and a viral infectivity complementation assay. Sequencing and functional analyses of the nef gene from 1988 are in progress. Analysis of the accessory gene region revealed the presence of inactivating mutations in two-thirds of clones recovered from 1988 and 1993. These data suggest that this patient, initially infected with a virulent swarm of HIV-1, is presently infected with a more attenuated viral quasispecies as a consequence of effective host immunoregulation. The researchers feel that an intense immunologic and virologic study of long-term survivors of HIV infection will provide a blueprint for understanding host immunoregulation and for dissecting out the range of HIV genotypes associated with an attenuated clinical course. The latter studies have direct relevance to live attenuated vaccine development.

"Broadness of Seroreactivity to the V3 loop of HIV-1 Correlates with the Emergence of V3 Genotypic Variation in Infected Patients"-

Project Description:

Recent data has suggested that the degree of viral quasi species diversity reflects the intactness of the host immune response. The researchers investigated the relationship between epitope-specific immune responses and viral quasispecies evolution in ten patients enrolled in a Phase I safety trial of gp160. Humoral responses to the HIV_{LAI} V3 loop were sequentially determined over a 3 to 4 year interval. DNA sequences corresponding to the V3 loop were also determined.

Significant Findings:

Six patients showed either a seroconversion or a boost in titer to the V3 reagent and a concurrent average of 3.3 amino acid changes in their autologous V3 loops. In contrast, 4 patients with little or no change in titer to the V3 loop showed an average of only 0.75 amino acid changes. Therefore, these data suggest that the broadness of host immune responses to viral epitopes are reflected in the rate of evolution of their cognate coding sequences. These data support the view that the host immune response to HIV-1 results in the continuous selection of new viral variants during the course of disease.

"Naturally Occurring Genotypes of the HIV-1 Long Terminal Repeat Display a Wide Range of Basal and tat-Induced Transcriptional Activities"-

Project Description:

The primary body of information on the structure of HIV-1 LTR/*gag* leader genotypes has been determined from the analysis of co-cultivated isolates. Functional studies of this regulatory portion of the provirus have been derived from the study of *in vitro* generated mutations of laboratory-adapted molecular clones of HIV-1. The researchers performed a longitudinal analysis of molecular clones from the LTR/*gag* leader region amplified directly from the peripheral blood of 4 patients over 3 years.

Significant Findings:

A remarkable number of point mutations and length polymorphisms in cis- and trans-acting regulatory elements were found within this cohort. Most of the length polymorphisms were associated with duplications of Sp1 and TCF-1a sequences. These mutations were associated with a wide range of transcriptional activities for these genotypes in a reporter gene assay. Mutations in conserved

Sp1 sequences correlated with a diminished capacity of such genotypes to bind purified Sp1 protein. Although no generalized trend in transcriptional activity was seen, a single patient accumulated mutations in NF-kB, Sp1, and TAR elements over this period. The analysis of naturally occurring mutations of LTR genotypes provides a means to study the molecular genetic consequences of virus-host interactions and to assess the functional impact of HIV therapeutics.

"Negative-strand RNA transcripts are Produced in Human Immunodeficiency Virus Type 1 Infected Cells and Patients by a Novel Promoter Downregulated by Tat"-

Project Description:

Current understanding of human immunodeficiency virus type-1 (HIV-1) transcription is based on unidirectional expression of transcripts with positive strand polarity from the 5' long terminal repeat (LTR). The researchers now report HIV-1 transcripts of negative strand polarity from acutely and chronically infected cell lines by use of a template orientation specific reverse transcription-polymerase chain reaction assay.

Significant Findings:

These observations were confirmed in natural infection by analysis of RNA derived from peripheral blood mononuclear cell samples from 15 HIV-1 infected patients. A cDNA derived from a 2.3 kb polyadenylated HIV-1 RNA of negative strand polarity was isolated from acutely infected A3.01 cells which encodes a highly conserved 189 amino acid open reading frame antiparallel to the envelope gene. Through the use of reporter gene constructions, it was shown that a novel negative strand promoter (NSP) functions within the negative response element of the 3' LTR which is downregulated by co-expression of Tat. Site-directed mutagenesis experiments demonstrated that NF-kB I and USF sites are crucial for NSP activity. These data extend the coding capacity of HIV-1 and suggest a role for antisense regulation of the viral life cycle.

"HIV-1 Proviral Genotypes from the Peripheral Blood Mononuclear Cells of an Infected Patient are Differentially Represented in Expressed Sequences"-

Project Description:

The RNA genome of the human immunodeficiency virus type 1 (HIV-1) is established as proviral DNA in infected cells. Only some of these cells may actively produce the array of viral RNAs which support progeny virion production. *In vivo* expression of a subset of proviral genotypes could influence

the experimental characterization of the viral quasispecies. The researchers explored the relationship between DNA and cDNA genotypes of the envelope gene by the molecular cloning and nucleotide sequencing of these templates from non-cultivated peripheral blood mononuclear cells from an HIV-1 infected patient.

Significant Findings:

Eleven proviral DNA and nine cDNA clones representing the V1 through V3 region of gp120 were recovered and sequenced. The proviral group was more heterogeneous than the cDNA group by nucleotide sequence changes and V1 length polymorphisms. Deduced amino acid sequences from this data set revealed that the two groups were distinct by primary structure, by the position of N-linked glycosylation sites, and by the net charge of the V3 loop. The V1-V2 region discriminated between the groups more strongly than the V3 region. The differential representation of HIV-1 envelope genotypes in the cDNA versus the proviral compartment may have important implications for the pathogenesis of disease and for the design of antiviral therapeutics.

"Correlation between Monoclonal Antibody Binding Kinetic Constants and Neutralization of HIV-1. "-

Project Description:

This project measured both association and dissociation rate constants between human mAbs specific for the third hypervariable (V3) region and recombinant gp120. These data were then compared to the ability of these mAbs to neutralize homologous HIV-1.

Significant Findings:

Monoclonal antibodies specific for the V3 loop of HIV-1 were capable of neutralizing laboratory strains of HIV-1 *in vitro*. In this project the researchers demonstrated, using surface plasmon resonance and biosensor technology, that the ability of V3 specific mAbs to neutralize HIV-1(MN) correlated with the dissociation rate constant of the homologous mAb-gp120 binding interaction. MABs capable of binding diverse strains of gp120 with similar association rate constants exhibited marked differences in the dissociation rate. The dissociation rate, and not the association rate, was found to be predictive of the neutralization capacity of the mAb. Furthermore, synthetic peptides corresponding to the V3 loop of HIV-1(IIIB, MN) yielded quantitatively similar binding kinetic profiles abrogating the need for purified recombinant gp120 protein and potentially facilitating the screening of more diverse isolates. Biosensor immobilized V3 peptides were found to mimic their conformational structure in solution. This

offers advantages to peptides studied by ELISA where some degree of denaturation and masking of epitopes can occur upon adsorption of peptides to plastic surfaces. The impact of amino acid substitutions within epitopes on subsequent mAb binding was dissected by observing alterations in dissociation rates. The technique provides rapid kinetic analyses of V3 antibody binding interactions with diverse V3 sequences facilitating the efficient screening and selection of those most likely to possess the broadest and most potent HIV-1 neutralizing potentials.

"Development of a Serotyping Assay to Differentiate Individuals from Thailand Infected with either an HIV-1 Isolate Belonging to either Genotype B or E"-

Project Description:

There are at present at least seven distinct genotypes of HIV-1 worldwide with as much as 30% variation in DNA sequence between genotypes. Within each genotype (clade) family there is 15-20% diversity while within a single HIV-1 infected individual there is inpatient diversity as great as 5-10%. These quasi species mutate over time within a given individual potentially allowing for variants capable of evading immune surveillance. Serologic assays capable of identifying predominate genotypes within particular geographic regions or infecting a particular individual may be relevant for the selection of optimal prophylactic or therapeutic HIV-1 vaccine candidates.

Significant Results:

The research demonstrated that BIAcore™ measurements of HIV-1 serum binding reactivity to peptides corresponding to the third hypervariable (V3) region of gp120 serologically distinguish the two predominate HIV-1 genotypes in Thailand. V3 sequences were determined through PCR amplification and DNA sequencing of HIV-1 isolates from individuals infected with both HIV-1 genotypes. Multiple V3 peptides were synthesized and rapidly screened to select optimal peptides for use in BIAcore serology. Serotypes were also distinguished based upon BIAcore reactivity to whole recombinant HIV-1 envelope glycoprotein (gp160) corresponding to prototype genotype B and E viruses. In addition, preferential serum reactivity to genotype B or E gp160 correlated with the ability of these sera to neutralize HIV-1 primary isolates of the homologous genotype indicating that the serotype might also be predictive of immunotype.

"Dependence of gp120 Conformational Structure on Binding to Epitope-Specific Monoclonal and Polyclonal Antibodies. "-

Project Description:

This project's purpose was to identify the presence of CD4-binding incompetent gp120 molecules within preparations of recombinant 'native' gp120. The researchers developed an assay, using a biosensor matrix and surface plasmon resonance, which rapidly and reproducibly measures antibody reactivity to HIV-1 gp120 in various structural conformations. In particular, antibodies displaying preferential reactivity to a CD4-binding competent ('native', rgp120) or CD4-binding incompetent ('reduced', rcmgp120) monomeric gp120 molecule were distinguished. This technique has advantages over conventional ELISA methodology where it is both difficult to control the concentration of protein adsorbed to the ELISA wells and a significant disruption of protein structure occurs upon adsorption.

Significant Findings:

A population of gp120 molecules which lacked CD4 receptor binding capacity and bound antibodies specific for 'reduced' gp120 was found within several 'native' gp120 preparations. The relative amount of this CD4-binding incompetent population varied among the various preparations studied. This presence of CD4-binding incompetent molecules within various 'native' recombinant gp120 preparations might have implications for HIV-1 envelope vaccine development. By measuring antibody binding ratios, several mAbs were identified which although elicited by immunization with various 'native' gp120 preparations, bound specifically to 'reduced' gp120. The ability to screen antibody specificity against HIV-1 envelope proteins with different conformations will assist in determining the quality of antibodies induced by various HIV-1 envelope vaccine candidates.

"Comparisons Between the Immune Responses Elicited by Candidate HIV-1 gp120/160 subunit Vaccines and Natural Infection"-

Project Description:

This project studied the comparisons between the immune responses elicited by candidate HIV-1 gp120/160 subunit vaccine and natural infection and then correlated these observations with the ability of vaccinee sera to neutralize divergent laboratory-adapted HIV-1 and primary HIV-1 isolates.

Significant Findings:

Vaccination with some preparations of both gp120 and gp160 elicited antibodies that were qualitatively similar in their preferential reactivity to a reduced, denatured gp120 protein. Only a fraction of these antibodies were capable of cross reactivity with the oligomeric gp120/gp41 complex expressed on the surfaced HIV-1 acutely infected cells. This contrasted with the antibody profile induced during natural infection where preferential binding to native monomeric gp120 protein and strong cross reactivity with the oligomeric gp120/gp41 complex is observed. This qualitative difference in antibody profiles induced during gp120/gp160 vaccination and natural infection might explain the ability of HIV-1 sera, but not these vaccinee sera, to neutralize primary HIV-1 isolates in a PBMC based assay. The presence of these vaccine-induced, denatured specific antibodies might be attributable to several factors including the presence of denatured antigen within the preparation, denaturation of the antigen upon formulation with adjuvant or the processing of antigen *in vivo*. The researchers extended these studies to examine the impact product variables such as strain, adjuvant formulation and dose schedule have on the induction of native specific antibodies.

"Determine the Role HIV-1 Serum V3 Antibodies Play in Neutralizing Primary HIV-1 Isolates"-

Project Description:

It was recently observed that immune sera generated by many candidate subunit HIV-1 vaccines were not capable of neutralizing primary isolates of HIV-1. These observations enhanced research efforts to assess which antibody populations may be lacking in these immune sera as well as determining which are critical in mediating primary HIV-1 neutralization in order to more effectively evaluate candidate HIV-1 vaccines.

The objective of this project was to compare the role of HIV-1 serum V3 antibodies in neutralizing primary HIV-1 isolates to V3-mediated neutralization of laboratory-adapted strains of HIV-1.

Significant Findings:

To identify epitopes important in neutralizing primary HIV-1 isolates, the researchers selectively depleted sera of antibodies specific for various epitopes within gp160 and assessed their functional consequences. Simultaneously, the researchers sought to gain insight into the differential mechanisms of neutralization involved in primary versus lab-adapted viral isolates. Amino acid sequencing of the V3 loop region from PBMC along with contemporaneous viral stocks were determined for 3 HIV-1 infected patients, the peptides synthesized

and then used for serum V3 depletion. Depletion of HIV-1 sera with a V3 peptide (clade B) depleted V3 antibodies (>95%) binding to multiple clade B peptides to include those with changes within the GPGX tip of the loop. Depleted and undepleted sera were studied for their ability to neutralize HIV-1_{MN} and two primary HIV-1 isolates in a viral infectivity reduction assay. While the majority of HIV-1_{MN} neutralization was lost upon V3 depletion, minimal impact on the neutralization of primary isolates was observed. This suggested a fundamentally different role for V3 antibodies in mediating neutralization in these two assays and more importantly implicated antibodies with epitope specificity outside of V3 as being major determinants in the neutralization of primary isolates. The researchers extended these studies by depleting HIV-1 sera of antibodies to other linear regions of gp160 as well as to monomeric and oligomeric gp120/gp160 complexes to determine the relative role of oligomeric or conformational-specific antibodies in the neutralization of primary isolates.

"Characterization of a Soluble Secreted Oligomeric gp160 Molecule"-

Project Description:

Current monomeric HIV-1 gp120/gp160 subunit vaccines were unable to elicit in seronegative human volunteers an immune response capable of neutralizing primary HIV-1 isolates. This project sought to characterize protein complexes which were reactive with sera from HIV-1 infected individuals.

Significant Findings:

Using BIAcore™, the researchers characterized a novel, oligomeric gp120/gp41 protein complex, produced in milligram quantities from chronically infected cells, that was highly reactive both with sera from HIV-1 individuals and oligomeric-specific antibodies.

"Phenotypic and Genotypic Analysis of Virus Isolates from HIV-infected Children with Rapid versus Slow Disease Progression" -

Project Description:

This project represented a collaborative effort between the Pediatric Branch of the National Cancer Institute and the Immunoregulation Program Area which was established to seek correlates of disease progression which might be applicable to vaccine therapeutic trials. In this study, pairs of isolates from infancy and after two years of age were compared in 8 HIV infected children.

Significant Findings:

Half of the patients demonstrated rapid progression to clinical immunodeficiency and 3 of the 4 have expired. The remaining four patients have been clinically stable over a similar interval of evaluation. Viral isolates were obtained through peripheral blood derived PHA lymphoblast co-cultivation (PBMC), titered on PBMC and compared at equivalent MOI in syncytia induction in MT-2 culture and ability to infect monocyte monolayers. In addition, the isolates were sequenced in envelope regions putatively required to confer syncytia induction and monocyte tropism. All infant isolates grew well but the later isolates exhibited wide variation in growth kinetics with slow growing isolates found in children with stable CD4 numbers. All four slow progressors had non-syncytia inducing isolates at follow-up even though two had intermediate strains in infancy. Three of four rapid progressors had SI isolates. Monocytotropic viruses were present in 7/8 patients (the exception was a rapid progressor) in infancy but disappeared in two of the remaining three rapid progressors but was retained by all slow progressors. The genotypes of the isolates from each patient were distinct by phylogenetic analysis (that is they resided in separate branches of the phylogenetic tree). However, comparison of early and late isolates from the same patient failed to disclose significant changes in any parameter of V2 or V3 (i.e. charge, length, etc.) even if the phenotype changed. Genotypic evaluation of the patients confirmed the presence of positively charged amino acids in the V3 loop conferring SI phenotype when the entire pool of isolates sequenced are compared. However, no evidence was found to support the contribution of the V3 loop to SI phenotype.

"Comparative Susceptibility to Serum Neutralization of Viral Isolates Derived from Lymph Node, Plasma and Peripheral Blood" -

Project Description:

Genotypic and phenotypic characterization of HIV-1 from different tissues revealed evidence of compartmentalization of viral quasispecies potentially reflecting the effect of immune selection and/or host cell tropism. In particular the advent of drug resistance in virus derived from plasma prior to its appearance in peripheral blood suggested that plasma quasispecies represent the pool of selective pressures. The relationship of plasma quasispecies to tissue proviral populations and humoral immune responses was not well defined. The researchers sought to characterize the genotype and phenotype of env in plasma, peripheral blood (PB) and lymph node (LN) from a pediatric patient on AZT. A context for the comparison was provided by analysis of 5 additional time points, three of which included plasma and viral stocks as well as provirus.

Significant Findings:

Virtually no difference in env sequence (V1-V3) diversity was observed between LN and contemporaneous peripheral blood provirus and the three compartments (PBMC, LN and plasma) were more similar to each other than to other time points. LN stocks were very similar to contemporaneous proviral and plasma populations but stocks derived from peripheral blood were as different from these species as clones from a subsequent time point. The isolates from LN, plasma and PBMC were evaluated in an infectivity reduction assay to determine their susceptibility to serum neutralization. The plasma isolate, and to a lesser extent, the LN isolate, were more susceptible to neutralization than the contemporaneous PBL isolate (13.7 vs 6.7 vs 1.9 fold reductions respectively). Additional LN/plasma/PBL samples are under evaluation to determine the generality of this observation.

"Mechanism and Therapeutic Implications of Transcriptional Competition Between HIV Isolates"-

Project Description:

Using the polymerase chain reaction to discriminate between HIV_{LAI} and HIV_{RF}, the researchers had shown previously that chronically-infected ACH2 and H9/IIIB cells (HIV_{LAI}) could be superinfected with HIV_{RF} and that the frequency of superinfection increased with time. Transcription of the superinfecting HIV_{RF} exceeded that of the host HIV_{LAI}, a phenomenon we have labeled transcriptional dominance. The mechanism of HIV_{RF} transcriptional dominance, however, was not apparent. In contrast, superinfection of ACH2

with a hybrid HXB2-neo^R provirus was not detectable by DNA PCR until G418 was added to enrich for cells containing the neo^R gene, suggesting that the ability to propagate progressively in culture may be HIV-strain specific. Southern blot analysis suggested that both the host and superinfecting viruses were integrated and grossly intact.

Significant Findings:

Clones of ACH2 superinfected with HIV_{RF} (ACH2/RF) were obtained through limiting dilution. Clonal ACH2/RF cells showed a predominance of HIV_{RF} transcription similar to that seen in bulk populations; PMA induction of the superinfecting virus (HIV_{RF}) occurred more rapidly than the host provirus. Similarly, after PMA stimulation of ACH2/HXB2-neo^R clones, transcription from the superinfecting strain (HXB2-neo^R) was more rapidly induced. 5-azacytidine had little effect on the transcriptional relationship between HIV_{LAI} and HIV_{RF}; increases in transcription were seen in both viruses. Sequencing of PCR-derived LTR fragments from A3.01 cells acutely infected with HIV_{RF} or from ACH2 cells showed differences in the U3-R region. The fragments were inserted upstream of the CAT gene. There was, however, little difference in transactivation potential between HIV_{RF} and HIV_{LAI} LTRs. Therefore, full-length Tat cDNAs from ACH2 and an acute A3.01/HIV_{RF} infection were obtained by RT-PCR, sequenced, and cloned into expression vectors. Prototypical Tat proteins were then tested against the LTR fragments from HIV_{LAI} and HIV_{RF}. Tat from HIV_{RF} was consistently more transcriptionally active than Tat_{LAI}. Interestingly, TAT_{RF} is 17 aa longer than Tat_{LAI} and more closely resembles "wild-type" Tat. The increased transcription of HIV_{RF} and, by extension, the temporal increase in the prevalence of dually-infected cells in culture might be related to the greater activity of Tat_{RF}. These data have implications for the modification of host provirus gene expression by replication-competent or -defective HIV-based vectors.

"Cytokine Enhancement of anti-HIV Immune Responses"

A. IL-7 and IL-12 Augmentation of Cellular Immunity

Project Description:

The cellular immune response to HIV plays a critical role in the control of HIV-1 infection. IL-7 and IL-12 have previously been shown to have potent effects on the generation of proliferative T-cell responses to antigen. To dissect the relationship between the anti-HIV cellular immune response, IL-7 and IL-12, the researchers concurrently examined the effect of HIV gp160 vaccination, IL-7

and IL-12 on the anti-gp160 cellular immune response in HIV-infected patients. Peripheral blood mononuclear cells from 20 HIV-infected patients vaccinated with gp160 and 20 patients receiving a placebo were cultured with IL-7 or IL-12 and either tetanus toxoid (TT) or gp160.

Significant Findings:

HIV infection was associated with a restriction of the cellular immune response to envelope; a minority of HIV-infected patients had cellular proliferative responses to gp160. Vaccination with gp160 increased the number of patients who had proliferative responses to gp160, relieving the restriction of cellular immune responses imposed by HIV infection (Figure 1). Both cytokines augmented proliferative responses to gp160 (See Figure 2). IL-7, but not IL-12, induced new antigen-specific proliferation from placebo recipients. IL-2, IL-4, IL-6, IFN γ and TNF α levels in supernatants of stimulated cultures showed no correlation with proliferation. HIV p24 production in culture was infrequent, and there was no consistent effect of IL-7 or IL-12 on p24 production. These data demonstrated that the cellular immune response against HIV can be positively affected by IL-7 and IL-12. Vaccination strategies that incorporate IL-7 or IL-12 as adjuvants, supplied exogenously or produced endogenously, might selectively boost cellular reactivity to HIV.

B. Cytokine Gene Transfer into HIV-Specific T-cells

Project Description:

Cytokines participate in a complex, reciprocal interaction between the immune system and HIV. The researchers previously demonstrated that IL-7 augments HIV-specific immune responses. A murine retroviral vector expressing both the IL-7 gene (in the sense or antisense orientation) and the gene for neomycin resistance (neo^R) was created and transfected into a murine packaging cell line. Retroviral supernatants from packaging cell lines containing sense or antisense IL-7 genes were used to infect and transduce the IL-7 gene into tetanus toxoid- (TT) and HIV gp120(MN)-specific T-cell lines.

Significant Findings:

Quantitative PCR showed that the number of cells containing the neo^R gene increased to nearly 100% with serial passage in geneticin. IL-7-specific mRNA was detected only in T-cells receiving the sense IL-7 gene and increased with passage in geneticin. Following stimulation with antigen-pulsed, irradiated, autologous PBMC, TT- and gp120-specific lines transduced with the sense IL-7 gene produced up to 898.4 pg/ml of IL-7; mock-infected and antisense IL-7 transduced cells made no IL-7. Independent of IL-7 production, all antigen-specific cell lines made IL-4, IL-6, and gIFN, peaking 2-5 days poststimulation; there were no significant differences in cytokine profiles between T-cells

subjected to mock infection or infection with either the sense or antisense IL-7 gene. Following antigenic stimulation, IL-7 production peaked later (day 7) and was of greater duration than IL-4, IL-6, or gIFN. TT-specific T-cells stably expressing the IL-7 gene showed higher TT-specific proliferation than mock-infected or antisense-IL-7-gene-containing cells (See Table 3). Taken together, these data suggested that strategies to augment immune activity against HIV by transduction of cytokine genes into antigen-specific T-cells might be feasible and that IL-7 might be an attractive candidate cytokine for these studies.

"Evaluating Crossreactive T-lymphocyte Epitopes within gp120 in HIV Uninfected Individuals" -

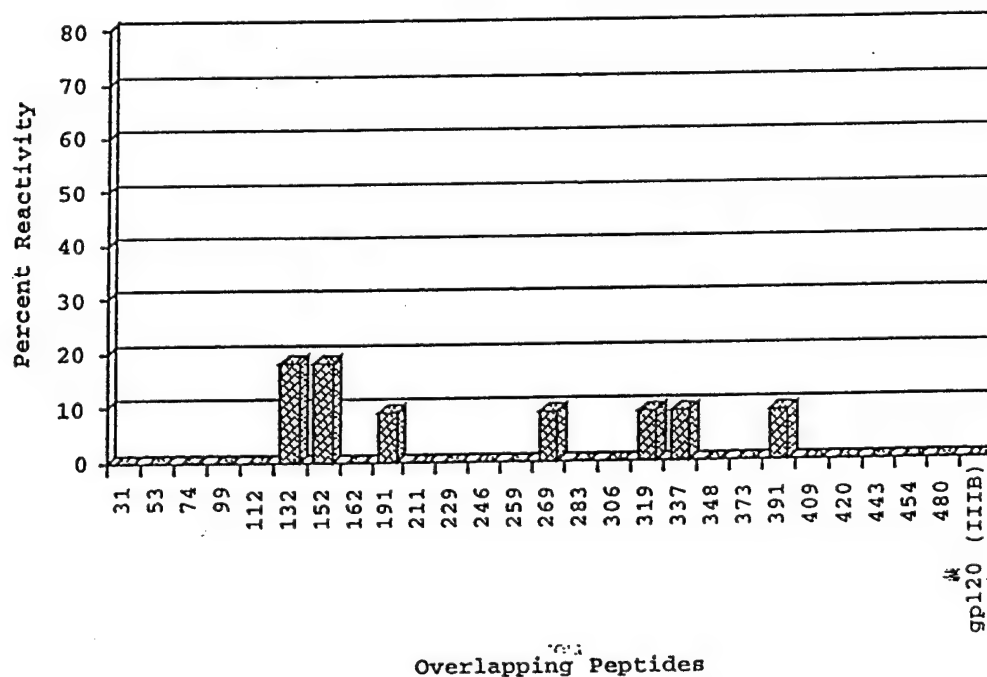
Project Description:

PBMCs were incubated from HIV uninfected volunteers with overlapping peptides covering the gp120 molecule which allowed for the determination of crossreactive proliferative T-lymphocyte epitopes. In order to determine whether crossreactive T-lymphocyte epitopes exist in HIV uninfected individuals, PBMC based epitope mapping was performed.

Significant Findings:

11 HIV uninfected controls have been studied to date, with the following preliminary results:

GP120 Peptide Reactivity BY PBMCs From Normal Controls



"Mapping the T-lymphocyte Epitopes in gp120 from PBMCs from HIV-infected Patients" -

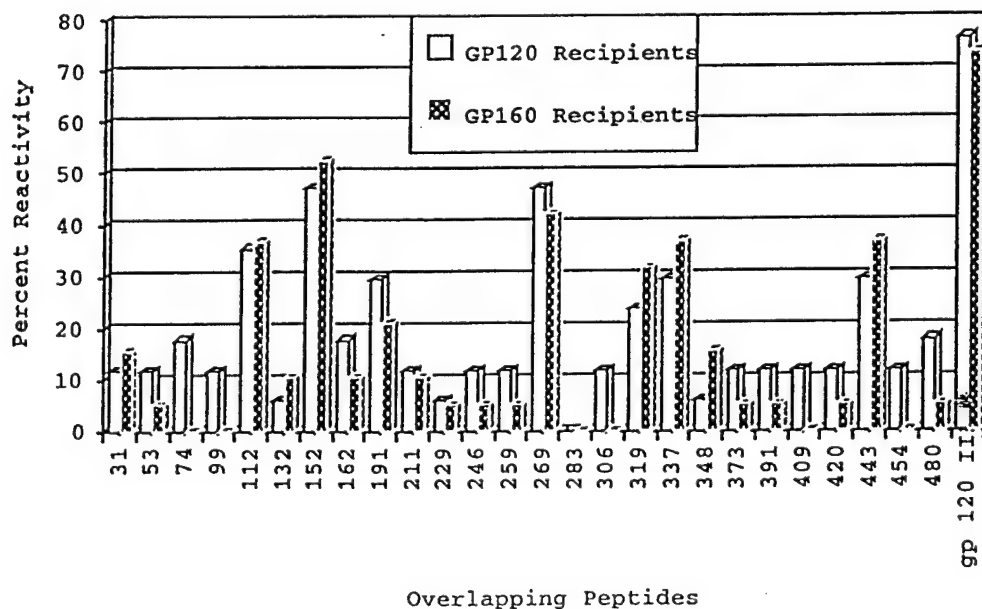
Project Description:

Incubating PBMCs from HIV infected participants in the RV21 and RV51 trial with overlapping peptides covering the gp120 molecule allows the determination of proliferative T-lymphocyte epitopes. In order to determine whether specific T-lymphocyte epitope reactivity, or patterns of reactivity, were related to immunoregulation of HIV infection, epitope mapping must be performed in a cohort of infected individuals. To further elucidate whether different envelope vaccine products induced different cellular immune reactivity, a cohort of both gp160 and gp120 recipients were utilized.

Significant Findings:

19 gp160 vaccine recipients and 17 gp120 vaccine recipients have been studied to date, with the results to be presented in November 1994.

GP120 Peptide Reactivity By PBMCs: Comparison Of GP120 And GP160 Recipients



"In vivo T-lymphocyte Mapping (RV98)" -

Project Description:

Using peptides in a DTH reaction, the purpose of this project was to attempt to map the T-lymphocyte epitopes *in vivo*. Two patients had their epitopes within the V3 loop well defined using their CD4 lines. To determine whether the same epitopes can be measured using a DTH reaction, these individuals will have peptides containing their previously defined epitope and other non-epitope sequences injected into their epidermis. Sequential evaluations at 24, 48, and 72 hours will determine whether there was a specific reaction. Peptides utilized for this project will be submitted for an IND from the FDA in January 1995. Planned enrollment into the trial is anticipated in early to mid 1995.

Significant Findings:

The human use protocol associated with this project has been approved; work will be initiated in early 1995.

RV21 I Naive vs Memory Phenotyping

Project Description:

Flow cytometric analysis was performed at ~6 month intervals of routine and CD45 isotype phenotypes. This project tested the hypothesis that memory (CD45RO) T-lymphocytes may be preferentially infected by HIV-1. In addition, there was speculation of a defect in the ability of HIV infected patients' T-lymphocytes to transition from the naive to memory phenotype. This study compared the phenotypes of a subgroup of HIV infected patients (selected for "vigorous" early response) at varying time points in a gp160 vaccine trial to similar HIV infected patients who were in the pre-immunization stage of a gp120 vaccine trial. The objective was to determine whether there were significant differences in the vaccine recipients as compared to their approximately matched controls.

Significant Findings:

12 patients from the Phase I gp160 protocol and the Phase I gp120 protocol were studied - (patient numbers 1, 8, 10, 13, 15, 16, 17, 19, 20, 29, 32, & 33)
Analyses were performed in Fall '91, Spring '92, Fall '92, and Spring '93

"RV51 Naive vs Memory Phenotyping" -

Project Description:

Flow cytometric analysis was performed at each protocol blood draw of routine and CD45 isotype phenotypes to test the hypothesis that memory (CD45RO) T-lymphocytes may be preferentially infected by HIV-1. In addition, there was speculation of a defect in the ability of HIV infected patients' T-lymphocytes to transition from the naive to memory phenotype. This study assessed the basic and CD45 isotype phenotypes of 27 patients in a gp120 vaccine trial from pre-immunization until the completion of the trial. The objective was to determine whether there were significant changes in the phenotypes over the course of the gp120 immunization series.

Significant Findings:

Acquisition of analyzed data continues on the 27 patients (patient numbers 28-60, excluding 32) analyzed prospectively from ~September 1991 to the present.

"RV21 II b and c Phenotyping" -

Project Description:

Flow cytometric analysis was performed at each protocol blood draw of routine and, in a random subset, experimental phenotypes were performed to test the hypothesis that monitoring phenotype changes over the course of gp160 or placebo immunization may provide insight into the pathophysiology of HIV infection as well as changes associated with repeated gp160 immunization.

Significant Findings:

Acquisition of data continues with >3,000 samples processed over the past year.

"Establishment and Evaluation of CD4+ T-Lymphocyte Lines from HIV-1 Seropositive Patients" -

Project Description:

CD4+ T lymphocyte lines were developed from eleven HIV-1 seropositive volunteers enrolled in a double-blind, placebo controlled, phase II, gp160 vaccine therapy trial. Lines were grown from PBMC collected 150 days post immunization with gp160 or placebo.

Significant Findings:

To date, nine out of twenty of the specific T cell lines were challenged against a panel of overlapping peptides spanning the gp120 LAI sequence. The response was heterogeneous, the most recognized peptides were LAI 74 in the C1 region and LAI 306 in the V3 region. In order to evaluate the proliferative capabilities to heterogeneous strain of HIV-1, the specific lines were challenged against a panel of divergent HIV-1 envelopes [gp120MN, gp120SF2 (env 2, 3) and gp160CM (clade E)]. Fifty-five percent of the lines were able to cross-recognize gp120MN while only 22% cross-reacted with gp120SF2. Only the gp160 specific lines cross-reacted with gp160CM. Peptide LAI 74 seems to be the common epitope recognized by the cross-reacting lines. To address a functional property of these T cell lines, cytotoxic assays were performed. B-LCL pulsed with antigen or peptides were killed by 3/3 of the specific lines tested. Supernatants from the 9 lines were assayed for presence of p24. Two out of nine lines were positive for p24 with high antigen titer which did not seem to interfere with their functional properties.

"HIV-Envelope Specific CD4+ T-Lymphocyte Lines Developed from Two HIV-1 Seropositive Patients Recognize Different Epitopes on the V3 Loop"-

Project Description:

In this project a novel epitope was described for the first time situated on the left side of the V3 loop and different envelope proteins developed in different expression system generated T-lymphocyte lines with the same epitope specificity. CD4+ lines were developed from seropositive patients #2 and #3 using either gp120III B (Genentech) and gp160III B (MGS). Both gp120 and gp160 specific lines developed from the two patients proliferated in response to the 25aa V3 III B loop (NNTRKSIRIQRGPGRAFTIGKIGC).

Significant Findings:

To define the epitope(s) recognized by the lines, 2 sets of peptides were constructed, one bearing serial truncations of three aa from the amino terminal and the other bearing the three aa truncations from the carboxyl terminal of the 25aa V3 peptide. The truncations were terminated at 9aa length. Both gp120 and gp160 lines from patient 2 and the gp160 line from patient 3 were challenged with the V3 truncations. For both lines from patient 2 the epitope was defined as RKSIRIQRG on the left side of the loop while for the patient 3 gp160 line the epitope was defined as AFVTIGKIG on the right side of the loop.

"Mapping CD4 T-Lymphocyte Epitopes in a Long Term Survivor"-

Project Description:

Distinctive immunologic and virologic characteristics of long term survivors may contribute to the development of therapeutic and preventive vaccines. A clinically and immunologically stable (CD4 count >500 x 4 years) adolescent who was perinatally infected with HIV 13.5 years ago is undergoing extensive evaluation of viral phenotype, genotype and virus specific immune responses.

CD4 T-lymphocyte lines were established from a single time point and were used to map the reactivity to various HIV envelope peptides. Peripheral blood mononuclear cells (PBMC) obtained by Ficoll density centrifugation were subjected to 3 cycles of stimulation, 15 days apart, with rcmgp120 IIIB and rcmgp120 MN. Each line was tested for specificity using a 3 day proliferation assay. In addition, the lines were challenged with various HIV envelope proteins and reactivity was assessed using a 3 day proliferation assay. Viral burden was measured with quantitative PCR and env sequences were determined by PCR cloning of a 736 nucleotide fragment containing V1-V3.

Significant Findings:

Viral burden in the PB at age 13.5 years was at the lower limit of detection (1-10 copies/100,000 cells). Env sequences were highly restricted with less than 1.5% total diversity. CD4+ T cell lines were successfully established with rcmgp120 IIIB and rcmgp120 MN with lymphocyte stimulation indices (LSI) of 49 and 62, respectively. The gp120 IIIB line cross-reacted with the gp120 MN molecule (LSI=14) while the gp120 MN line cross-reacted with the gp120 IIIB molecule (LSI=20) as well as a northern Thai strain,

gp160 CM, (LSI=4). The gp120 IIIB line also reacted to peptides in the C2 and C5 region when challenged with 11 peptides partially covering the gp120 sequence.

Preliminary mapping of the CD4 T lymphocyte gp120 epitopes from a long term survivor were determined. The gp120 lines cross-react with gp120 molecules of different viral genotypes and further epitope mapping reveals reactivity to a previously undescribed region of C2.

"Mucosal Immunity to HIV-1"-

Project Description:

The sera of 33 HIV-1-infected individuals, previously shown to neutralize HIV-1_{MN} *in vitro*, were screened by ELISA for IgA reactivity against rgp120_{MN} and a synthetic V3_{MN} loop peptide. Six were selected for evaluation of the effect of serum IgA from infected individuals on the *in vitro* infection of susceptible target cells by HIV-1_{MN}. Using protein G immobilized on sepharose, the researchers depleted the sera of IgG to a level undetectable by nephelometry and viral envelope-specific ELISA. The IgA component of the IgG-depleted serum was affinity-purified with immobilized jacalin, a lectin which selectively binds the IgA1 fraction of human immunoglobulin.

Significant Findings:

IgG-depleted sera and purified IgA1 serum fractions showing IgA reactivity against rgp120_{MN} and V3_{MN} by ELISA inhibited the *in vitro* infection of CEM-ss cells by HIV-1_{MN}, but sera depleted of both IgG and IgA1 did not. These data show that, like serum IgG, serum IgA from selected HIV-1-infected individuals were capable of neutralizing HIV-1_{MN} *in vitro*. The biological significance of this observation and the identities of serum IgA-recognized HIV-1 neutralization epitopes remain to be determined.

Parotid saliva and colostrum of HIV-1 seropositive subjects have been assayed for oligomeric gp160-binding IgA and IgG. The parotid saliva of some HIV-1 infected individuals was found to have low titer oligomer-binding IgA not correlating with serum IgA envelope antigen-binding titer. The colostrum of 4 of 4 HIV-1 infected females demonstrated both IgG and IgA oligomer-binding activity, and preliminary data suggests that colostrum sIgA, prepared by lectin affinity purification, is capable of neutralizing HIV-1(MN) *in vitro*, although with much lower efficiency than unfractionated lipid-free colostrum supernatant from the same subject. Goals for FY 95 include further functional and antigen-binding

analyses of colostral immune responses to HIV-1 and a comparison of mucosal immune responses to HIV-1 in parotid saliva, nasal secretions, and genital secretions.

"Cross-Reactivity between Clades of Serum from HIV-1 Infected Individuals or Envelope-Immunized Rabbits" -

Project Description:

The investigators synthesized sets of overlapping 12-mer peptides which cover the entire sequence of the HIV-1 envelope from various clades, including: B clade (lab isolate LAI/LAV, MN, SF2 and wild-type isolates US3 from North America and BK132 from Thailand), A clade (DJ263), C clade (DJ259), D clade (SE365), E clade (NT235) and F clade (BZ126). These peptides were used to investigate the linear antibody response from rabbits immunized with HIV-1 gp160, either recombinant or affinity purified from infected cell cultures.

Significant Findings:

Antibody reactivity elicited by both immunogens was broadly reactive and was strongest in regions of the envelope that have previously been characterized as "constant" by genetic typing. Antibodies against the following epitopes recognized sequences from all 6 clades were investigated: 6 in gp120 including 3 in C1, 1 in C2, 1 in C3, 1 in C5 and 2 in gp41. Cross reactivity of sera from patient infected with viruses of the following genotypes was then investigated: B-clade (from the US), B-clade (from Thailand), and E-clade from Thailand. Regions of the envelope previously characterized as immunodominant using samples from B-clades proved to be immunoreactive in the E-clade samples, as well. Reactivity was directed against the constant regions crossed clade boundaries in these samples as well.

"Microencapsulation of HIV-1 Envelope Glycoprotein"-

Project Description:

A new protocol was written to assess the structural and functional effects of encapsulating HIV-1 envelope glycoprotein in poly(DL-lactide-co-glycolide) (PLG) microspheres and to investigate the feasibility of using such a formulation as an HIV subunit vaccine. The structure and function of the glycoprotein released from loaded microspheres will be evaluated by measuring binding to CD4 and against a panel of monoclonal antibodies recognizing either discontinuous or linear epitopes. HIV seronegative rabbits will be immunized systemically (IM), and postimmunization sera will be assayed quantitatively and qualitatively for immunogen-binding antibodies. These sera will also be assayed for their capacity to neutralize HIV-1 *in vitro*, specifically addressing cross reactivity within and between clades. Cellular immune responses will be measured by *in vitro* CTL assay of spleen cells from intraperitoneally (IP) immunized inbred mice.

Significant Findings:

Not applicable at this time.

Projects in the Biochemistry/Serology Laboratory

Technical Approach

"Antibody Purification"-

Techniques were developed to quantitatively remove antibodies versus the V3 loop from infected patient sera. Numerous depletions were performed. They played into the following studies:

1. Antibodies vs. V3 loop play a different role in neutralization of primary isolates than they do in neutralizing lab strains.
2. Researchers were trained to perform these studies, allowing comparison of depletion of antibodies vs. Thai E and US B strains.

Serology

Accomplishments:

Began administration on a new laboratory with new functions. These new functions are being brought on-line:

1. ELISA's: B-2 microglobulin assay, Tetanus assay (for trials)
2. Immunoblots: Quality checking previous results, assay of HIV-1 peptides (for NLM's V3 study, for George Lowell's adjuvant study)

PEPSCAN

Accomplishments:

1. Completed Phase I PEPSCAN and immunoblot study.
2. Developed new technique allowing use of serum at much higher dilution (to be presented at the International Peptide Symposium next Spring).
3. Completed Britta Wahren adjuvant study.
4. Completed George Lowell adjuvant study.
5. Manufactured and testing of peptides against 6 Clades of HIV-1 (A-F, with 2 against E, and 4 against B).

Spectrotyping

Accomplishments:

1. Phase I study published.
2. Collaboration established to study antibody binding to V3 loop peptides. This study has recently been broadened to include measurement of affinities between V3 loop peptides and antibodies against them.

SIV Serology

Accomplishments:

1. E11S blocks (PEPSCAN) were made and Quality controlled. They established the pattern of seroreactivity of infected monkeys (both with E11S and 251).
2. ELISA's based on biotin-labeled peptides binding to strepavidin have been developed. These are being used to quantitate seroreactivity of immunized monkeys. The ability to measure binding to peptides 88 and 500 (previously not possible) was established.

Peptide mapping strategies

Accomplishments:

Peptides were made and purified covering the entire sequence of gp120(LAI). Two complete sets were made:

1. 30-mers overlapping by 10 and 12-mers overlapping by eight. These were used successfully to map T-cell lines by investigators in this Program Area.
2. Peptides were made and are now in the process of being made under Good Laboratory Practice Conditions as skin-test antigens for a proposed human use protocol.

IV. CONCLUDING REMARKS

The Immunoregulation Program Area has utilized its availability of prospective clinical samples and its ongoing development of unique laboratory reagents to critically position itself to have the ability to rapidly dissect *in vivo* HIV immunoregulation and to define the correlates of effective immunoregulation. Presently over 700 volunteers are enrolled in ongoing vaccine trials with nearly 2,500 patients years accrued. These trials have yielded valuable insights into HIV immunoregulation and have predicted the immune responses induced by current immunogens within seronegative cohorts. The long term follow-up of early Phase I studies, gp160 and gp120, have allowed the dissection of humoral and cellular epitopes in natural infection and the identification of additional recognition sites induced by immune manipulation. In addition, the correlation of virologic parameters with immune response, though preliminary, are providing insights into HIV immunoregulation as well as the virologic consequences of induced immune pressure. These insights have value not only in the development of innovative treatment strategies for HIV-infected persons but also will be instrumental in designing strategies for preventive vaccine developments.

This Program Area also demonstrated its flexibility by quickly responding to a major shift in trial execution responsibilities. With the withdrawal of Wyeth-Ayerst from these trials, this Program Area rapidly responded to the major transition and strategically reorganized its resources to accommodate a major work load increase for the trial monitoring and execution. The current execution model of this trial with concurrent data platform design will serve as a model and blueprint for planned trials in other Program Areas. It is worthwhile to note that even with this transition, the gp160 Phase II trial continues with a remarkably low missed visit rate of 1%.

Summarily, the reagents, technologies, and expertise developed in this program area have greatly contributed to the progress of the HIV research program and has also been of significant benefit to the scientific community at large. As a direct measure of productivity, this program area published 38 articles during this reporting period.

V. PUBLICATIONS AND PRESENTATIONS

A. MANUSCRIPTS

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INTERVENTION ASSESSMENT

USAMRMC Program Area Coordinator:

Maryanne Vahey, Ph.D.

HJF Scientific Director:

Kenneth Wagner, D.O.

Program Area Summary:

The objectives of the Intervention Assessment Program Area focus on the development and evaluation of methods for rapid diagnosis and quantitation of HIV as well as the exploration of studies of methods to assess interventions. Surveillance of HIV incidence and disease progression in military populations are also areas of focus, emphasizing longitudinal natural history studies.

Human Use protocols

Protocol Number	Title (Abbreviated)	Principal Investigator
RV 1	Natural History	Wagner
RV 2	Core Diagnostics	Wagner
RV 5	Dental Natural History**(Completed)	Konzelman/Winn
RV 22	Retrospective Review	Oster
RV 44	Syphilis(Completed)	Johnson
RV 67	Staph Aureus**	Decker

* Funded by the National Institute of Dental Research

** Funded by a pharmaceutical firm

Laboratory Work Units

(Project Titles abbreviated)

I. Technology Reduced to Practice

High Throughput Liquid Hybridization PCR

Direct Detection Fluorescent Readout PCR

II. **Clinical and Field Trial Support**
Preventive Vaccines Program Area

Development of RT-PCR assay for Clades B and E

Initiation of Development of Quantitative LH-PCR Assay System for SIV

Processing of 60 DNA/RNA Template Preparations for Hybridization Mobility Assay

HIV Disease Prevention Program Area

Development of LH-PCR Assay and Template Preparation Systems for LN

Refinement of PCR Technique for Determination of 215 Mutation

Completion of PCR analysis for RV43 Resistance Study

Immunoregulation Program Area

Development of PCR method for Determination of NEO Gene Levels

Intervention Assessment Program Area

Characterize the Dynamics of Viral Influx and Afflux

Characterize Natural History of Disease Progression in Cohort of 100 Women

III. **San Antonio Facility**

Natural History Support

Expansion of Loss to Follow up

Retrieval and Confirmation of Clinical Data for Rapid/Slow Progressor Cohort

Identification and Tracking of Concordant Couples

Section of Applied Immunology

Evaluate Usefulness of Archived PBMCs for Phenotypic Marker Analysis

Identify Antigen-Specific T cells in PB Using Flow Cytometry

Develop New and Novel Cellular Assays of Immune Function

Applied Molecular and Cell Biology

Complete Viral Load Characterization in Slow/Rapid Progressor Cohort

Initiate *in vitro* Assay to Assess Viral Infectivity

Develop Strategy for Amplification of Full Length Sequences

I. OVERVIEW

The Intervention Assessment Program Area is divided into two main components, a clinical program and a laboratory program. Within the clinical program, several longitudinal studies comprise the primary work. Two natural history protocols, RV1 and RV2 collect, collate and store primary and longitudinal clinical specimens and data from HIV-infected persons across the three services. The data bases within these two protocols currently track over 4500 patients and serve as the population from which other clinical trials within the program derive cohorts. The data assembled on these protocols constitutes the central repository for the surveillance of HIV disease in the military population. These protocols are now in the sixth year of data collection and are approaching the point where enough endpoints have been collected so that statistically meaningful data can be generated. Clinical investigative efforts supported by these protocols include:

1. Documentation of HIV seroconversion dates
2. Documentation of all AIDS defining endpoints and death
3. Maintenance of a strong quality assurance program for collected data
4. Assessment of racial, sex and age issues in the natural history of HIV
5. Assessment of background incidence of certain diseases for use as historical controls.
6. Provision of samples for investigative laboratory efforts.

The laboratory efforts are organized into three work units. The processing and applications units are located in the 1600 E. Gude Dr., Rockville, MD facility and the development and investigative units are located at Wilford Hall Medical Center, San Antonio, TX. The primary work unit is provision of the assessment of the clinical and field trials of the MMCARR through the utilization of validated surrogate markers. A second work unit supports the efforts in practical assay development that will ultimately support applications in the clinical and field trial work unit. Twenty percent of the program area's laboratory effort is devoted to assay refinement and technical validation of methodologies to assess newly defined surrogate markers. The third work unit employs the natural history protocols RV1 and RV2 as a basis on which to formulate hypotheses. Employing well-defined study cohorts as models for the analytical studies of the natural history of HIV disease, this unit focuses on progression within the paradigm of defining what clinical, immunologic and virologic surrogate markers are prognostic of the ultimate disease course and outcome. Emphasis is placed on the derivation of new surrogate markers of HIV disease by scrutiny of the patterns of such markers in the natural history setting, with the goal being the ultimate application of such methodologies to the assessment of clinical trials.

II. RESEARCH GOALS AND OBJECTIVES

In support of the primary mission of the HIV Research Program, the reduction and prevention of HIV incidence among military medical beneficiaries, this program area has the following objectives:

- A. Manage and consolidate the clinical data bases and specimen repositories of the tri-services.
- B. Derive and characterize new markers of progression in the natural history of HIV disease.
- C. Develop new methodologies to accurately assess quantitative and functional markers of HIV disease progression.
- D. Support clinical and field trials through the application of validated immunologic and virologic methods for the assessment of HIV disease progression.

III. TECHNICAL APPROACH

The Intervention Assessment Program Area achieves its goals and objectives through a series of natural history studies and laboratory projects. These protocols and laboratory projects are described below:

Clinical Studies

RV1-"The Natural History of HIV Infection and Disease in United States Military Medical Beneficiaries"-

Protocol Description:

The intent of this study is to systematically document the natural disease progression of HIV infection in military medical beneficiaries. RV1 is the primary natural history study and is now into its sixth year of data collection. Information is collected during each staging visit for the HIV infected patient and maintained in a database. The information is then utilized for multiple purposes and protocols. Unique aspects of the database include documented clinical and laboratory data, stored serum and cells, significant number of seroconverters, and a racially mixed population with equal access to health care which serves as a reference source for other research Program Areas and protocols.

Significant Findings:

As of 29 July 1994, 3070 patients were enrolled in the Natural History Study (RV1). Enrollment was 215 patients for RV1 during this reporting period. A primary goal for this 12 month reporting period was the concerted effort to find the lost to follow-up (LTFU) patients (patients not returning within 18 months) or locate their medical record data if they had expired or went to other facilities. Other goals were to perform intense quality control on the collected data, ensure all data was present on enrolled patients (especially endpoints), and identify the serum samples that can be linked to the natural history data. Tremendous gains were made in all the above stated goals. For example, WRAMC was able to complete 148 of the 172 LTFU(96 since Oct 93) patients who were signed up for RV1, had died, and had no longitudinal data (this took approximately 8-40 hours/pt). Also, at WRAMC, LTFU patients have dropped from 30% to 5%. At NNMCC 35 of 51 LTFU patients were found for re-evaluation. The Air Force was able to retrieve a large number of death certificates and have information or scheduled evaluations on 41 of their 99 patients who were LTFU. They now have death data on 64 of 124 patients deceased.

Comparing the computer based data (laboratory and clinical) to hard copy generated data at 10% per month per center has made the data base incredibly accurate for the many who use it.

Patients Enrolled: 2128
New enrollments during reporting period: 215

Site(s): Walter Reed Army Medical Center, Washington, DC
National Naval Medical Center, Bethesda, MD
Wilford Hall Air Force Medical Center, San Antonio, TX
Brooke Army Medical Center, San Antonio, TX

RV2-"Core Protocol for HIV Developmental Diagnostics (Adult)"

Protocol Description:

The purpose is to develop and evaluate new and/or improved laboratory methods for establishing the diagnosis of HIV infection and for determining the stage of illness. Various methods are utilized and developed to detect replicating HIV virus, HIV antigens and HIV nucleic acids. Methods include virus culture, antigen capture immunoassay, and polymerase chain reaction (PCR) amplification of HIV DNA.

Significant Findings:

The Core Diagnostics Protocol (RV2) has been under heavy scrutiny to determine where each serum specimen is located and to set up a data link that will allow ready determination of quantity and quality of specimens. Although this is an ongoing effort, great gains have already been made. Specimens have been located and are catalogued for easy retrieval. The ability to request and locate a specimen is at about an 80-90% level. The linking of the data bases between the storage facility and the HIV Research Program Database is ongoing.

Number of Patients Enrolled: 1825
New enrollments during reporting period: 191

Patient Visits: 1201

Site(s): Walter Reed Army Medical Center, Washington, DC
National Naval Medical Center, Bethesda, MD
Wilford Hall Air Force Medical Center, San Antonio, TX
Brooke Army Medical Center, San Antonio, TX

RV5-" The Natural History of the Oral Manifestations of HIV Infection in a U.S. Military Population"-

Background:

Previous reports of the Oral Manifestations of HIV infection were published by the VA and the epidemiology of specific oral conditions by John and Deborah Greenspan of the University of California at San Francisco and Michael Glick of Temple and the University of Pennsylvania. However, all such reports were based on study populations whose HIV infections were diagnosed on the basis of symptoms and therefore the majority were in an advanced stage. The value of the military patient population was the fact that they were largely found seropositive by military screening programs and therefore were mostly asymptomatic and in the early stage of HIV disease. About 2/3 of this population were asymptomatic and 1/3 in Walter Reed stages 5 or 6. They therefore gave a superior cross-sectional sample of the spectrum of HIV infection and its oral manifestations.

Protocol Description:

The purpose of this protocol was to determine the prevalence, incidence and risk factors for oral manifestations of HIV infection with relation to the degree of immunodeficiency. This study was designed to develop longitudinal data based upon biannual examinations. It is unique in that the patients represented a wide range of early to late stage HIV-1 infection. The protocol was initiated in April 1989 at the Walter Reed Army Medical Center. Volunteers received a comprehensive oral and dental examination at study entry and every six months thereafter. The evaluation included clinical examinations for periodontal diseases, dental caries, and oral mucosa conditions. Samples of saliva and subgingival dental plaque were collected at each visit for microbial and biochemical assays, and a questionnaire on oral health related behavior was administered.

Significant Findings:

The Dental Natural History Study discontinued accessioning data in July 1994. Over the next year analysis of data will take place at the National Institute of Dental Research at the National Institutes of Health, Bethesda, MD. These comments below are based on examiners clinical impressions and not on data analysis:

In general the oral health of the volunteer subject population was superior to most comparable non-military cohorts. This was largely due to the system which requires periodic exams and demands a certain level of good oral health for mobilization preparedness. Although dental treatment is mandated only in terms of crisis, nonetheless dental commanders are urged to implement

programs geared to enhance the oral health of their target population. The overall quality of care is good; it is accessible to active duty members and it is without cost to the individual. The investigators saw very few, probably less than 12 %, in poor dental repair.

Even more significant was the impression that the oral hygiene and therefore the oral health of our subjects was improved from visit to visit and certainly over the duration of the protocol; this occurred despite progress of the HIV disease. Data analysis may confirm less plaque and calculus. The reason for oral hygiene improvement may simply be the frequency of exam which would emphasize oral health. It may also represent the desire of the volunteer to earn approbation from the dental research team. The personal responses of the volunteers lends credence to this impression. Virtually each subject, after each exam thanked the dental staff for their quality and thoroughness of procedure, the effort to help them with oral health needs and their positive attitude and caring. The procedure was invariably described as a positive experience.

Another impression to be verified by data was the high percentage of cigarette smokers in our population and the pathogenic effect of smoke on the oral soft tissues.

Number of Patients Enrolled: 1073

Patient Visits: 402

Site(s): Walter Reed Army Medical Center, Washington, DC

RV22-"The Clinical Presentation of HIV Infected Patients at Walter Reed Army Medical Center"-

Protocol Description:

This protocol was designed to evaluate clinical and laboratory data on the first 400 adults seen in the WRAMC clinic who are infected with HIV-1 by retrospectively reviewing their records.

Significant Findings:

This retrospective protocol is being conducted concurrently with the RV1 Natural History study. 50 charts were reviewed in the last year.

An addendum was submitted during the past year requesting to extend the duration of this protocol for 5 years, ending in 1998.

Number of Patients Enrolled: Not applicable

Patient Visits: Not applicable

Site(s): Walter Reed Army Medical Center, Washington, DC

RV44-"The Effect of HIV Infection on the Initial Manifestations and Response to Treatment of Syphilis"-

Protocol Description:

This protocol was a collaborative effort with the CDC and Medical Centers at WRAMC, NNMCM, Philadelphia, Baltimore, Brooklyn, San Francisco and Houston. This study was conducted to assess whether HIV infected patients are at increased risk of failing treatment for early syphilis and to assess the effect of an enhanced therapy regimen. It was a multi-center, randomized, double-blind, treatment trial comparing usual therapy (Penicillin G benzathine 2.4 mg I.M.) with enhanced therapy (usual plus amoxicillin 6 g/d and probenecid 1.5 g/d for 14 days).

Significant Findings:

This protocol was closed to enrollment December 31, 1993; data analysis continues.

Number of Patients Enrolled: 11

Site(s): Walter Reed Army Medical Center, Washington, DC
National Naval Medical Center, Bethesda, MD

RV67-"A Placebo Controlled Double-Blinded Study of the Elimination of Staphylococcus Aureus Carriage in HIV infected Patients with Topical Antimicrobial agents "-

Protocol Description:

The purpose of this protocol is to determine the efficacy of topical Antimicrobial agents, mupirocin calcium ointment and chlorhexidine gluconate 4% foam in the eradication of S. aureus Nasal and skin carriage in HIV seropositive patients.

Significant Findings: Protocol in progress, no data available.

Number of Patients Enrolled: 99

Site(s): National Naval Medical Center, Bethesda, MD

Laboratory Component

The Intervention Assessment Program Area - Laboratory Component achieves its objectives through the conduct of laboratory projects which utilize clinical specimens from ongoing human use studies in three program areas. These projects are briefly described below:

"Derivation and Characterization of New Markers of Progression in the Natural History of HIV Disease" -

Significant Findings:

Two significant study cohorts have been defined, the rapid/slow progression cohort and the women's cohort. Extensive characterization of clinical, virologic and immunologic markers in these populations is underway.

"Technical Development of New Methodologies to Accurately Assess Quantitative and Functional Markers of HIV Disease Progression" -

Significant Findings:

The LH-PCR assay for quantitative viral load determination has been filed for patent. Significant progress has been made in the development of the fluorescent direct detection PCR methodology.

"Support of Clinical and Field Trials through the Application of Validated Immunologic and Virologic Methods for the Assessment of HIV Disease Progression" -

Significant Findings:

The PCR laboratory in Rockville has processed over 4000 samples to include specimens for study in: RV21, RV43, RV77, RV78 and RV91.

1. Technology Reduced to Practice

a. High Throughput Liquid Hybridization PCR
DOD Patent Pending # 08/154416 12/15/93

b. Direct Detection Fluorescent Readout PCR
Invention Disclosure anticipated by 9/30/94

2. Clinical and Field Trial Support

a. Preventive Vaccines

- development of a differential RT-PCR assay for clades B and E
- initiation of development of a quantitative LH-PCR assay system for SIV
- processing of 60 DNA/RNA template preparations for hybridization mobility assay
- processing 60 differential RT-PCR clade B & E

b. HIV Disease Prevention

- development of quantitative LH-PCR assay and template preparation system for tissue sections, whole tissue and fine needle aspirate preparations of lymph node materials
- refinement of the nested PCR technique for the determination of the 215 mutation in HIV infected persons
- completion of the quantitative PCR analysis of plasma and/or serum viral load in all patients in the RV-43 trial which demonstrated that the viral load marker significantly determined disease course and outcome

c. Immunoregulation

- development of a quantitative PCR method for the determination of NEO gene levels to be used to track gene therapy vectors

d. Intervention Assessment

- initiation of efforts to characterize the dynamics of viral influx and efflux in the periphery to elucidate the relationship of viral load measures made on plasma, serum and PBMCs to the status of the germinal centers

- initiation of efforts to characterize the natural history of HIV disease progression in a cohort of 100 women

San Antonio Facility

Section of Natural History Protocol Support

1. Expansion of the loss to follow up tracking of HIV infected Air Force personnel.
2. Retrieval and confirmation of clinical data for the 70 patient cohort of rapid and slow progressors.
3. Continuation of identification and tracking of pairs of concordant HIV infected couples for possible laboratory studies (28 pairs identified to date).

Section of Applied Immunology

1. Initiation of efforts to evaluate the usefulness of archived PBMCs for phenotypic marker analysis (to broaden the evaluation of frozen specimens).
2. Initiation of efforts to identify antigen-specific T cells in the peripheral blood using flow cytometry in place of the lengthy traditional MHC restricted cytotoxicity assays.
3. Initiation of efforts to develop new and novel cellular assays of immune function in HIV infected persons.

Section of Applied Molecular and Cell Biology

1. Completion of the characterization of viral load in the PBMCs and serum of HIV infected persons in a cohort of 70 rapid and slow progressing individuals.
2. Initiation of the derivation of an *in vitro* assay to assess viral infectivity that will employ quantitative viral load evaluation.
3. Development of a strategy for the amplification of full length viral sequences from primary patient material for subsequent RFLP and viral complexity analysis.

IV. CONCLUDING REMARKS

The Intervention Assessment Program Area has made great progress towards technology development. The Rockville PCR facility was designated as an ACTG reference laboratory for quantitative PCR. The flow cytometry laboratory at the Wilford Hall Medical Center was designated as a DAIDS reference laboratory for cell sorting and phenotypic analysis. The White House AIDS Coordinator made a site visit to the San Antonio facility in May, 1994. In acknowledgment of her expertise, the Program Area Coordinator was invited to co-author a full length chapter on LH-PCR methodology to the Cold Spring Harbor Book on Methods in PCR. The Natural History studies continue to offer the program and the scientific community at large a tremendous resource of longitudinal data which will provide valuable insights into the natural history of HIV.

V. PUBLICATIONS AND PRESENTATIONS:

A. MANUSCRIPTS

Michael N, Vahey M, D'Arcy L, Ehrenberg PK, Mosca JD, Rappaport J, Redfield RR. (Feb. 1994). *Negative Strand RNA Transcripts are Produced in Human Immunodeficiency Virus Type-1 Infected Cells and Patients by a Novel Promoter Downregulated by Tat*, Journal of Virology, 68, 2, pp. 979-987.

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B. ABSTRACTS:

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